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ERRATA AND AUTHORS' EMENDATIONS

Page 95, Table I, third line, should be tumbler "1C" instead of "2C"; sixth line, "2C" instead of "3C"; eventh line, "3A" instead of "1A."

Page 151, first column, line 17, "Loch Katrina" should be "Loch Katrine."

Pages 190, 191, and 192. For "blue mold" read "green mold" and for "green mold" read "blue mold."

Page 249, Table V, heading for section 2, the date should be April 20, 1918, not April 20, 1917.

Page 249, the last word in the first column should be "unfavorable," not "favorable."

Page 300, line 4 of legend, change "setting of sex organs" to "setting off sex organs."

Page 300, line 6 of legend, change "470 diameters" to "940 diameters."

Page 306, delete period following "branches" in line beginning "A" in legend.

Page 310, first column, line 5, change "binmoia" to "binomial."

Page 411, first column, line 28 from the bottom, delete hyphen in "hyphae-bearing."

Page 594, Figure 1 should be reversed.

Page 775, last line of legend, "soild" should be "solid."

Page 793, first column, line 2 from bottom, "tonkinensi" should be spelled "tonkinensis."

Page 810, first column, line 2 from bottom, the second "as" should be "at."

Page 815, first column, line 13 from the bottom, second sentence, "30°" should be "20°."

Page 824, Table III, in the fourth line of figures from the bottom, ".14" under "Gain" should be a loss of that quantity.

Page 836, second column, line 18 from the bottom, "ND_1" should be "C_1D_1."

Page 837, first column, line 24, "Arachonites" should be "Arachnites."

Page 842, line 3 from the bottom, "Spicarioldes" should be "Spicarioides."

Page 982, Table I, fourth column, "*a*" under Uteri should be "*a'*."

Page 990, Table I, column 5, line 2, "82.77" should be "82.70."

Page 1038, first column, line 23 from the bottom, "(Greef.)" should be "(Greeff)."

Page 1120, legend of fig. 8, line 2: "The yields generally lie between," etc. should read: "The yields after tobacco generally lie between those," etc.

Page 1150, second column, last paragraph, "The literature dealing with the chemistry of the European grape, *Vitis vinifera*, is in agreement that sucrose is never found in the mature fruit of this species." Since the proof sheets of this article were read, an article by Copeman (Copeman, P. R. v. Q. R., An investigation with some physical and chemical changes occurring in grapes during ripening. Science Bull. No. 30, Dept. Agric. Union So. Africa, 38 pp., 30 figs., 1924) has been received. Copeman analyzed four varieties of *Vitis vinifera*, Red and White Hanepoot, Barbarossa, and Flaming Tokai, at weekly intervals, beginning four weeks before ripening began and continuing three weeks after the fruit was fully ripe. Sucrose was present in all stages and increased in amount after full ripeness was attained. This is apparently the first report of the presence of sucrose in fully ripe *Vitis vinifera*.

Page 1161, second column, line 7 from the bottom, "as soon as" should read "as some."

Page 1161, second column, last line, add "days" to "one-half the number of."

Page 1166, first column, line 6 from the bottom, "that that" should read "that the."

JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXX WASHINGTON, D. C., JANUARY 1, 1925 No. 1

A STUDY OF VARIABILITY IN THE BURT OAT [1]

By FRANKLIN A. COFFMAN, *Associate Agronomist in Oat Investigations, Office of Cereal Investigations, Bureau of Plant Industry;* JOHN H. PARKER, *in Charge of Crop Improvement, Kansas Agricultural Experiment Station, and Agent, Office of Cereal Investigations;* and KARL S. QUISENBERRY, *formerly Assistant in Agronomy, Kansas Agricultural Experiment Station* [2]

INTRODUCTION

The oat crop is the third most important cereal crop in the United States, being outranked by corn and wheat only. There are two general oat-growing regions, one northern and one southern. White oats are most popular in the colder north, while red oats are better adapted to the warmer southern States.

Burt, a selection made from a commercial field of Red Rustproof about 1878, is one of the principal representatives of the varieties of red oats. The original selection is supposed to have been made by a man named Burt, whose name the variety now bears. The exact place of origin is not definitely known but is believed to have been in Greene County, Southern Alabama. It is probable that Burt, May, Early Ripe, and June are different names for the same variety.

The Burt oat is widely adapted and is commercially grown in the South and Southwest. It has long been recognized as a variable variety and its classification has been difficult. The variety contains strains resistant to crown rust, *Puccinia coronata* Corda, as well as strains resistant to loose smut, *Ustilago avenae* (Pers.) Jens., and covered smut, *U. levis* (K. and S.) Magn. The early maturity of Burt often enables it to escape injury from drought and rusts.

Recognizing the present economic importance and the potential breeding value of the variety, the writers undertook an extensive technical study to determine the nature and extent of its variability. As a necessary background for this investigation a wide review was made of the technical papers on oat classification and breeding. The variety proved to be far more variable, both in general characters and in kernel characters, than was previously realized.

Five characters have been studied, namely, spikelet disarticulation, floret disjunction, basal hairs, awns, and lemma color. Variability is found in all of these, as well as in general plant characters. The indications are that in some characters homozygosity may be attained, or at least approached, by pure-line selection. Association has been observed to exist between some of these kernel characters. No genetic analysis is attempted on the basis of the data presented.

The data presented in this paper, together with the literature review, are believed to be of especial interest to agronomists, geneticists, plant breeders, and botanists. They afford a basis for classifying not only the Burt oat but similar cultivated derivatives of *Avena sterilis* grown in the United States. Europeans long have designated the cultivated forms of *A. sterilis* by a separate specific name, just as both Europeans and Americans have desig-

[1] Received for publication July 18, 1924—issued February, 1925. Paper No. 154 of the Department of Agronomy, Kansas Agricultural Experiment Station. The investigations at Manhattan were conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Agronomy of the Kansas Agricultural Experiment Station. That part of the data herein reported which was obtained at the Akron Field Station was presented by the senior author to the faculty of the Kansas State Agricultural College as a thesis in partial fulfillment of the requirements for the degree of master of science, granted June 1, 1922.

[2] The writers wish to acknowledge their indebtedness to Dr. C. R. Ball, senior agronomist in charge; A. W. Warburton, formerly agronomist; and T. R. Stanton, agronomist, of the Office of Cereal Investigations, U. S. Department of Agriculture, for suggestions during the progress of the experiments and in the preparation of the manuscript; to Dr. H. H. Love, of Cornell University, for a critical reading of the manuscript; and to Supt. J. F. Brandon, of the Akron Field Station, Akron, Colo., for facilities provided.

nated those of *A. fatua*. It is believed that Americans should make a similar distinction in name between the wild or ancestral form, *A. sterilis*, and its cultivated derivatives. Of the many names used, the designation *Avena byzantina* Koch is believed to have priority.

The literature of the Burt oat is reviewed below under three main divisions, namely: (1) Importance and distribution; (2) description and classification; and (3) inheritance of spikelet characters in oats. In general the literature is discussed in the order of its publication.

LITERATURE ON IMPORTANCE

It has been shown by Parker (*100*) [3] and Durrell and Parker (*30*) that certain strains of Burt oats are highly resistant to crown rust (*Puccinia coronata* Corda). This character adds to the value of the variety, for, as Parker has shown (*102*), crown rust is widely distributed in the United States and may cause appreciable losses, particularly in the Southeastern States. The investigations of Norton (*99*), Zavitz (*159*), Reed (*109*) and Reed, Griffiths, and Briggs [4] show that certain strains of Burt and Early Ripe, a variety probably identical with Burt, are highly resistant to loose smut (*U. levis* (K. and S.) Magn.) and to covered smut (*U. avenae* Pers. Jens).

Burt is one of the earliest maturing varieties in the United States. This character often enables it to escape destructive rust infection and injury from drought.

For many years it has been recognized by those familiar with Burt oats that so-called pure-line selections from this variety very often do not breed true. Norton (*99*) was one of the first to recognize this fact. Parker (*101*) found that the strain of Burt oats used in his experiments was not homozygous for resistance to crown rust, although it was considered a pure line with respect to the usually observed agronomic characters. Several theories have been advanced to explain this phenomenon, but as yet no explanation based on experimental evidence has been offered.

During the past 20 years a number of oat crosses have been made in which the Burt oat was used as one of the parents. Some of these hybrids have been found to be of considerable promise. Results of varietal experiments conducted by Warburton, Burnett, and Love (*144*) at Ames, Iowa, Ithaca, N. Y., and certain other stations show that hybrids of Burt and Sixty-Day have given favorable results. Stanton (*123*) states that one of the highest-yielding varieties at the Fort Hays Branch Station, Hays, Kans., is a hybrid between Burt and Sixty-Day.

LITERATURE ON DISTRIBUTION

The distribution of the Burt variety in the United States is very wide (fig. 1). Probably few oat varieties have proved as well adapted over so wide a range of conditions. Records of varietal experiments conducted at experiment stations in nearly all States from northern Florida to southern New York and from the Atlantic coast to the Rocky Mountains and also in California show that comparatively favorable yields have been obtained from Burt oats, or hybrids between Burt and other varieties, in most of the States in this entire area.

For the convenience of the reader, the extensive literature reviewed is grouped geographically as follows: (1) General; (2) Southern States; (3) Northern States, Canada and Alaska; (4) Great Plains States and westward; and (5) South Africa and Australia.

GENERAL LITERATURE

Carleton (*16*) states that the early Burt is among the earliest varieties grown in this country and that it is an excellent sort simply because of its earliness, especially in districts where there is liable to be a large amount of rust or where the dry weather sets in early.

Warburton, Burnett, and Love (*144*) present data on oat varietal experiments and on breeding experiments with oats, conducted in many different States and under widely varying conditions. They report on experiments conducted in Illinois, Iowa, New York, Pennsylvania, Virginia, Ohio, Kentucky, and Indiana. At many of the stations in these States, Burt or hybrids between Burt and other varieties have outyielded all others, and at nearly all stations the Burt oat ranked comparatively high in yield.

Wheeler (*149*) includes the Burt variety among winter oats, and gives the following description:

> Stems half spreading in early growth, many per plant, heads like those of Red Rustproof; grains bearded, dull yellow, long pointed, with many short bushy hairs at the base; the grains hang together like those of Red Rustproof; ripens fairly early.

[3] Reference is made by number (italic) to "Literature cited," p. 60–64.

[4] G. M., Reed, Marion A. Griffiths, and Fred N. Briggs. Varietal susceptibility of oats to loose and covered smuts. U. S. Dept. Agr. Bul. 1275. [In press.]

In the list of desirable varieties for different States the Burt oat is commended for spring sowing in kansas, California, Florida, Kentucky, Mississippi, Nebraska, North rolina, Tennessee, Texas, Virginia, d West Virginia. The Burt oat is entioned also as a suitable winter oat Missouri, while for Oklahoma conions it is recommended as a spring t for most of the State and as a win- oat for the extreme southern border the State.

variety to use in reseeding badly winterkilled fields of oats.

Kilgore, Burgess, and Meacham (*62*) describe the Burt or Ninety-Day variety as one of the best, if not the best, of the spring oats for general sowing in North Carolina.

Garren (*44*) and Williams (*150*) of the North Carolina Agricultural Experiment Station found Burt one of the earliest oat varieties, and recommended it as one of the best for spring seeding under North Carolina conditions.

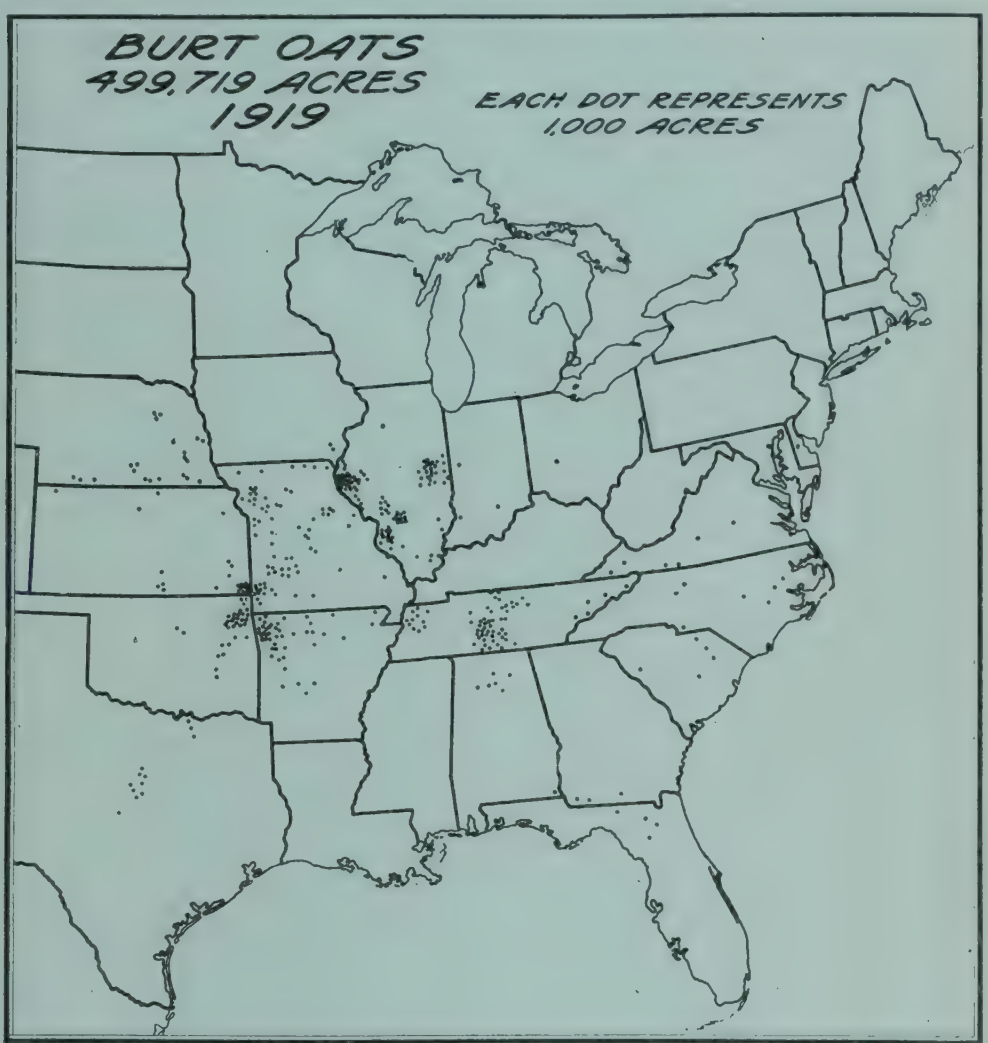


FIG. 1.—Map of the eastern and central United States showing distribution of the Burt oat in 1919

CONCERNING SOUTHERN STATES

Plumb (*104, p. 19*) states that Burt a locally grown variety that does well Mississippi, Alabama, Georgia, South arolina, and in central and eastern ennessee.

Warburton (*142*) states that the Burt at, sometimes called Ninety-Day or lay, occasionally is sown in the fall in he South. This variety is similar to ed Rustproof but is less hardy, and rdinarily is sown in the spring, as it sually does well from spring seeding. or this reason Burt is a desirable

Duggar and Cauthen (*29*) state that Burt often is preferred for seeding in the spring in all parts of Alabama, and that from Auburn southward Burt may be sown in the fall if seeded comparatively early. According to these authors Burt does not give as good results in Alabama as do some of the Red Rustproof strains.

Mooers (*80*) states that Burt has been the standard spring variety in middle Tennessee for a number of years and practically has superseded all others in the rest of the State. It is the earliest of the spring varieties,

and to this fact must be attributed its chief advantage, for it will make a fair yield when the later kinds fail for lack of moisture. Burt can not be used safely as a winter oat in Tennessee, as it is killed by even moderately cold weather.

Childs (*18*) observed that the Burt and Early Ripe varieties are very much alike. The kernels are long and lighter in weight and color than those of Red Rustproof. These varieties mature at about the same time as does Fulghum. One kernel of the spikelet is awned.

Nelson and Ruzek (*84*) found that Burt was not winter hardy enough to be dependable as a fall-sown variety in Arkansas, but that it was the highest yielding of the spring-sown varieties tested.

Nelson and Osborn (*85*) found that strains of the Burt variety generally figured among the highest yielders each year that spring-oat tests were made at Fayetteville, Ark. Burt strains led in yield during the 10-year period and were second only to Fulghum in a 4-year period. Burt has almost always produced grain of fairly good quality. Burt and Fulghum are said to be the best varieties for spring seeding in Arkansas.

Helm and Stadler (*48*) include Burt in the early spring-oat group, and recommend it as one of the varieties suitable for northern Missouri as well as for the central and southern parts of the State. They give the following description of this oat:

> A very early maturing variety of the Mediterranean group. It is somewhat spreading in early growth, though not so spreading as Red Rustproof. Under Missouri conditions the plants are taller and the heads longer than those of Red Rustproof. The head stem is not so straight as that of Red Rustproof and Fulghum. The grains are similar in form to those of Red Rustproof, and are bearded, but have short bushy hairs at the base and are dull yellow in color. At this station Burt oats have been the earliest maturing variety grown, maturing one to three days earlier than Kherson and Sixty-Day. They are remarkably resistant to smut and fairly resistant to leaf rust. Their yields have been high, and it is probable that they will be found a leading variety in this State.

Observations made by T. R. Stanton, and one of the writers (Parker) corroborate the statements of Helm and Stadler, at least for Clinton and Dekalb counties in northwestern Missouri. In company with R. O. Pixlee, of Cameron, Mo., they studied oat varieties on several farms in these two counties.

The Burt oat was introduced into this section of Missouri in 1888 or 1889 by James Chapman, who procured seed of Burt from his former home in Tennessee and sowed it on his farm about 2 miles west of Osborne, Mo. The Burt has been grown continuously since its introduction and the acreage has steadily increased until it probably is now grown more extensively than any other variety in this territory. Mr. Pixlee, who has been engaged in the elevator and grain business at Cameron throughout the period mentioned, has always encouraged the growing of Burt and has set aside seed of good quality for his customers. He has built up a special market demand for Burt for seed at the Kansas City and St. Louis markets, where several cars of Burt are sold each year, mostly for seed in southern territory.

The farmers interviewed who were growing Burt oat mentioned the following advantages of this variety: (1) Early maturity, (2) relatively high yields, and (3) freedom from smut. The fact that the Burt oat has maintained its place in this territory in competition with other varieties for more than 30 years indicates that it has characteristics which enable it to succeed under a wide range of soil and climatic conditions. This section of Missouri is not particularly well adapted to oats, and if it were not for the fact that Burt can be grown with a fair degree of success, the acreage and yield of oats in this section probably would be considerably reduced.

CONCERNING NORTHERN STATES, CANADA, AND ALASKA

Love (*67*) reports that the best combinations in oat crosses grown in New York are Burt with Texas Rustproof and Sixty-Day. In another paper Love (*68*) states that Burt and Sixty-Day hybrids and selections gave the best yields at Ithaca, N. Y., from 1907 to 1912.

Hickman (*51*), in reporting the results of varietal experiments conducted in Ohio in 1890 and 1891, refers to the varieties Rustproof and New Red Rustproof as "mixed" oats. Burt oats are believed to be a selection from Red Rustproof and the variable condition of Burt may be due to this recognized "mixed" condition in Red Rustproof.

Burnett (*13*) states that the Burt oat is popular in some of the southern Iowa counties, rivaling the Kherson in yield in sections where it is adapted.

Zavitz (*157*) reported that the Early Ripe (Burt) variety is the earliest oat among some 260 varieties grown at the Ontario Agricultural College. The grain is long and slender, giving a rather light weight per measured bushel. It seems evident that this variety is immune from the attack of smut.

he same author (*158*), in reporting n the results of a four-year study of the llering of oats, found that the Early ipe and Burt oats averaged 17 and 18 llers per plant, respectively, compared ith 14 and 17 for Sixty-Day and Kherson, respectively. However, avitz [5] states that Burt is not conidered of much agricultural value in ntario.

Georgeson (*45*), reporting on experiental work in Alaska, names Burt's Extra Early among the varieties rown. From the data he presents, he variety appears to be among the arliest maturing grown in Alaska. He states that oats are grown for hay nd make excellent silage.

CONCERNING GREAT PLAINS STATES AND WESTWARD

Jardine (*55*) in a paper on dry-land grains mentions the Burt oat as being one of the most promising drought-resistant varieties.

Stoa (*126*) states that red, gray, or black oats are not common in North Dakota.

Martin (*76*) has reported extensive nursery and plat experiments with oats on dry and on irrigated land in western South Dakota. Burt (C. I. No. 293)[6] produced an average acre yield of 33.7 bushels on dry land in the eight-year period from 1912 to 1919, inclusive. It was exceeded in yield by only one other variety, a selection of Sixty-Day, which averaged 36.1 bushels to the acre for the same period. Martin observed that the Burt variety as grown is a mixture of kernels of various colors.

Montgomery (*79*) found that Burt outyielded all other varieties at the Nebraska Agricultural Experiment Station. It was found, however, that the Burt lodged and rusted badly under unfavorable conditions. He states that the early oats grown at the Nebraska station are of either a reddish or yellow color, while the late varieties, with but one exception, are white. The early maturity of yellow oats also has been recognized by Zavitz (*159*) in the experiments conducted at the Ontario Agricultural College.

Snyder and Burr (*121*) state that of the varieties tested at the North Platte substation in Nebraska, Burt is one of the earliest and has ranked with Kherson in yield. It is said to be a few days earlier than the Kherson.

Kiesselbach and Ratcliffe (*58*) found that Burt outyielded all other varieties at the Nebraska Agricultural Experiment Station over a long period of years.

Ten Eyck (*128*) found the Burt oat to be one of the earliest varieties tested at the Kansas Agricultural Experiment Station. He stated that it was a very hardy and drought-resistant variety and recommended it for western Kansas.

Salmon and Parker (*117*) describe a high-yielding strain of Fulghum oats distributed by the Kansas station under the name Kanota. Data on the comparative yield of Burt oats are included in the same paper. In plats at the Agronomy Farm in the four-year period from 1917 to 1920, inclusive, a strain of the Burt oat (Kansas No. 5020) was outyielded only by Kanota, Fulghum (Kansas No. 6084), and a strain of Red Texas (Kansas No. 5205). In the nursery experiments, the average yield of Burt (Kansas No. 5020) for the five-year period from 1916 to 1920, inclusive, was exceeded only by that of Kanota.

Ross and Leidigh (*115*) found that of the varieties tested in the Texas Panhandle the Burt oat made the best yields, and stated that it was an extremely early brown variety, resembling Red Rustproof in some characteristics, but earlier. In another publication, Ross (*116*) describes the Burt as having smaller kernels than those of Red Rustproof, variable in color, ranging from yellowish brown to dark brown or almost black, and often distinctly striped. Burt matured a little earlier than the Rustproof group and produced slightly lower yields.

McMurdo (*74*) found that a selection of Burt, made by W. G. Shelley, and grown by McMurdo simply as Burt, was the highest yielding variety at the Akron Field Station in Colorado. This selection later was named Colburt by C. W. Warburton. Coffman [7] recommends it for seeding on dry land in northeastern Colorado.

Hendry (*49*) states that Burt is meeting with favor among California farmers. Trials at the University Farm, Davis, Calif., are said to have shown that this variety, which is described as gray seeded, possesses advantages over the red and black varieties in the interior districts which may warrant its release for general cultivation. In California, Burt is early,

[5] Letter to John H. Parker, 1916.
[6] Accession number of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Dept. Agr.
[7] COFFMAN, FRANKLIN A. CEREAL EXPERIMENTS ON THE AKRON FIELD STATION. U. S. Dept. Agr. Bul. 1287. [In press.]

possesses tall stiff straw, and fills early in the spring, often escaping the hot weather which stops the filling of the later-maturing varieties. Hendry thinks the Burt probably will not prove to be more prolific than the red or black varieties in the Coast districts and recommends it for only the interior of the State.

Florell (*36*) presents data on oat varietal experiments conducted at Chico, Calif. He states that—

> as a general group, the so-called red oats are preferable to all other types for the peculiarly hot, dry climatic conditions of the Sacramento valley.

He names Burt among the varieties of this group, but apparently does not consider it as one of the most important representatives of the group.

CONCERNING SOUTH AFRICA AND AUSTRALIA

Mundy (*81*) considers the Burt oat a variety of great promise in southern Rhodesia. It is said to be extremely rust resisting, producing a fine straw 2.5 to 3 feet in length, with heavy heads and grain of good quality. Seed of Burt is available in commercial quantities and can be purchased from South African seed merchants. He states (*82*, *83*) that the Burt oat did the best of all varieties under trial at the Arlington Sand Veld Experiment Station in Rhodesia. It was early and extremely vigorous but lacked the rust-resistant properties of Kherson. This latter observation is of interest, because in the article previously cited (*81*) he described Burt as extremely rust resisting, and in America it has been found to be more resistant to crown rust than the Kherson.

Archer and others (*4*) state that Burt is a good drought-resistant oat, suitable for conditions in West Australia and especially useful for early hay and early grazing.

LITERATURE ON DESCRIPTION AND CLASSIFICATION

The classification of the Burt oat has proved a difficult problem. Different strains have long been recognized as having varying characters and probably for this reason authors have classified Burt in different ways.

Duggar (*28*) and Duggar and Cauthen (*29*) describe this variety as follows:

> The majority of spikelets bear one bearded and one beardless grain, but some are double bearded and a few are entirely beardless. The grains are more slender than those of Red Rustproof oats, and are of a paler cream or brownish-yellow color. Most spikelets have only short bristles or none. The straw is taller and weaker than that of Red oats. The variety is tender and is adapted to spring sowing. It matures from 8 to 10 days earlier than the Red Rustproof oats. Burt and May are apparently the same variety.

Norton (*99*) states that—

> while *Avena fatua* has been considered the progenitor of the cultivated oats, some of the varieties, as the red oats of the South, are undoubtedly descended from *Avena sterilis*.

He believes it probable that a third wild species is represented by the common Burt oat of the Southern States.

Burnett (*13*) classes Burt as one of the typical varieties of Rustproof, which he groups with the red oats.

Carleton (*17*, *p. 97*) classifies Burt as belonging to *Avena sativa aristata* Krause, and describes the variety as "awned, hull white or light yellow."

Etheridge (*33*), in his classification of oat varieties, gives the following description of Burt:

> Culms semi-erect in early growth, otherwise similar to those of *Avena sterilis nigra;* sheaths, leaves, panicles, spikelets, and glumes similar to those of *A. sterilis nigra*, except that the glumes are shorter, ranging from 25 to 30 mm.; grains dull yellow, elongated, outer grains 18 mm. long, long-pointed; lemma glabrous, with 7 obscure nerves; awn usually present on the outer grain and frequently on the inner grain, seldom twisted; basal hairs usually present, numerous, short (1–2.5 mm.), fine; basilar articulation of the outer grain evident; rachilla of the outer grain short (2–2.5 mm.), strong, glabrous, persistent to the inner grain. Plants 5–8 dm. tall; medium early in maturing.

Etheridge obtained samples of Burt oat under the names Early Ripe and Red Rustproof. He classifies Burt as a variety of *Avena sterilis* and considers it one of the principal representatives of the red-oat group grown in the United States.

Waller (*141*) states:

> While the origin of some of the early maturing varieties is at present unknown, there is reason to believe that one at least, Burt, may have come from *Avena barbata*.

Warburton [8] believes that Burt probably is the result of natural crossing between varieties of *Avena sterilis* and *Avena sativa*.

Pridham (*107*) lists Burt and Early Burt among eight medium early maturing varieties tested in New South Wales and gives as one of the distinguishing characters that they closely resemble Algerian. He states that Algerian "is often of a pale brown tint in warm districts and dark brown in cool ones."

Archer (*4*), of Australia, also considers Burt a variety of *Avena sterilis*, and describes the variety as follows:

> Panicle, equilateral, spreading, erect, medium to long; spikelets, two grained, outer grain awned,

[8] Letter to F. A. Coffman, 1920.

asal articulation of outer grain very marked; umes, long, coarse, long pointed, nerves 9–10, fine; ngth of glumes 26 mm.; husk, pale yellow, gla- ous, nerves not prominent; awns, numerous, not ng, fine, neither twisted nor geniculate; basal hairs umerous, short, fine, white; rachilla, glabrous, ng, fine, persistent to upper grain; grain with husk uter grain) length 17–18 mm., long pointed, nar- w, practically no shoulder; grain without husk uter grain) length 8–9 mm., brush long; straw, ne to medium fine, non-hairy. First node of achis normal, straight, inclined to lodge; foliage, edium in width, medium to sparse, early growth, edium erect to medium spreading; stooling, parse; season, very early.

Marquand (*75*), of the Welsh Plant Breeding Station, considers the Burt oat a variety of *Avena sterilis*, subspecies *culta*, and gives the following description of the variety, which is in close agreement with that of Etheridge (*33*):

Young plant sub-erect. Lower leaves with marginal hairs towards the base of the lamina. Stems slender. Average height of the plant 80 cm. Panicle small, sub-equilateral, branches sub-patent. Spikelets 2–3 grained. Glumes 26–30 mm. long, 7–8.5 mm. wide. Grains dull yellow, attenuate; usually both upper and lower grains provided with long slightly twisted but rarely geniculate awns. Lower grain 17–20 mm. long, 2.5–2.8 mm. wide, 1.9–2.1 mm. thick; basal articulation only partly solidified. Outer palea glabrous, long pointed, with the apex not upturned. Rachilla also glabrous. Basal hairs numerous but very short (0.5–2 mm. in length), forming compact tufts. Early in ripening.

Marquand (*75*) also called attention to the fact, apparently not recognized by many agronomic workers, but emphasized in the present paper, that the majority of the older varieties of oats and some of the more recent ones are not pure lines but consist of a number of strains, sometimes amounting to hundreds, differing in their properties in a greater or less degree.

In a conversation with the writers in September, 1921, Prof. N. I. Vavilov, director of the Bureau of Applied Botany and Plant Breeding, of Petrograd, stated that possibly the Burt oat is similar to the European type classified as *Avena sterilis byzantina*.

Different authors do not agree on the amount of natural crossing which occurs in oats. It is generally believed, however, that while some crossing occurs, the extent is not great. Reports by Rimpau (*111*, *112*), Jamieson (*54*), Norton (*98*), Peacock (*103*), Livermore,[9] Heribert-Nilsson (*50*), Hayes and Garber (*47*), and others, indicate that natural crosses occur to some extent. Several other authors, namely, Hunt (*53*), Pope (*105*), Babcock and Clausen (*7*), Love and Craig (*72*), and Fraser (*37*) indicate that natural crosses in oats are of extremely rare occurrence.

From the results obtained in these experiments the authors believe that natural crossing in the Burt oat occurs to a greater extent than has been generally believed, although not so freely as believed by Jamieson (*54*). However, it probably is true that sufficient natural crossing does occur to account for some of the aberrant forms which appear and possibly for some of the recurring heterozygosity of the Burt oat.

Variability in oat varieties has long been recognized. More than 15 years ago Webber (*147*) stated that few pure strains of oats existed, and that varieties frequently were mixtures of different types. Warburton (*143*) pointed out the urgent need for definite experiments and scientific methods for oat improvement. Welton and Gearhart (*148*) have stated that great variability exists in the physiological and other characters of the plants of oat varieties. The extent and nature of the variability existing in Burt has only recently been fully realized, but the same condition may exist, to some extent at least, in other oat varieties.

AVENA BYZANTINA KOCH AND ITS RELATIONSHIPS

The following are brief reviews of the available literature on the classification of oats, with especial reference to the taxonomic position of the cultivated varieties supposed to have been derived from the wild *Avena sterilis*.

Koch (*63*, *p. 392*) published the original description of *Avena byzantina*, as follows:

Glaberrima; Spiculae magnae, biflorae; basis flosculi inferioris pilis quartam ejusdem flosculi partem attingentibus obsita, superioris nuda; Flosculi aristati, glabri, laeviusculi; Palea inferior bifida; Aristae pars inferior tortilis, glabra, laevis.

He states that it is similar in appearance to *A. sativa* but differs in having both florets awned and the lower hairy. He states further that Petermann's *A. hybrida*, according to its description, has a hairy rachilla and floret base. Koch based his description on plants from the vicinity of Constantinople and stated that he had received similar plants from the collection of Thirke made near Brusa and had previously determined these erroneously as *A. sativa*.

Cosson (*22*) published a paper on the classification of the species of Avena, in which *Avena byzantina* C. Koch is listed with *A. hybrida* Peterm. as being synonymous with *Avena fatua* var. *glabrescens*.

[9] LIVERMORE, K. C. NATURAL CROSS FERTILIZATION IN OATS. 1912. [Unpublished thesis, Cornell Univ., Dept. Plant Breeding.]

Thellung (*130*) published a very comprehensive paper on the origin, systematic position, and cultural history of the cultivated species of oats. He used Koch's name *Avena byzantina* for the cultivated varieties which have arisen from the wild *Avena sterilis*. A description of *Avena byzantina* is given, together with the synonomy. This species had been called *Avena sterilis* forma *parallela* by Haussknecht, *Avena sativa* var. *biaristata* by Hackel, and *Avena algeriensis* by Trabut. Thellung predicted that by using rational selection methods a true-breeding race having *sativa* characters could be produced from *byzantina*. He states that the biological and ecological relations of *Avena byzantina* also serve to distinguish this species from *Avena sativa*. The experiments of Trabut in Algeria and experiences in the Cape Province of South Africa, in Australia, and in the southern United States have shown that the varieties derived from *Avena sterilis* are best able to withstand the climatic conditions found in these regions.

Schulz (*119*) also uses the name *Avena byzantina* for the cultivated varieties which are supposed to have arisen from the wild *Avena sterilis*. *Avena byzantina* is said to occupy an isolated position. Most of the varieties of this species resemble those of *Avena sativa* in appearance but may be distinguished from it by the method of articulation and other characters. Schulz stated that *Avena byzantina* may be called the Mediterranean oat, as it occurs in Spain, Algeria, and Mesopotamia. It is said that oats of this kind were known by the Greeks and Romans.

Schulz (*120*) has written a history of the cultivated oats, including their nomenclature. He uses the name *Avena byzantina* for the cultivated polymorphous varieties of the Mediterranean group. He states that many forms of *Avena byzantina* closely resemble *Avena sterilis*, while other forms can scarcely be distinguished from *Avena sativa*. The *Avena algeriensis* of Trabut is considered synonymous with *Avena byzantina*.

Zade (*153*) gives a description of the kernels of *Avena byzantina* which are sometimes found in oat seed. He states that this species somewhat resembles the intermediate resulting from crossing between *Avena sativa* and *A. fatua* but that the kernels are longer and the form of the kernel base intermediate, a very unusual condition for the products of the crossing above mentioned.

Zade (*155*) carried on studies with oat species and varieties. He considers that *Avena byzantina* probably has arisen from *Avena sterilis*, as *Avena sativa* has arisen from *Avena fatua*. He states that this morphologically distinct species is a true intermediate between the cultivated and wild oats.

The papers quoted above show unmistakably that the name *Avena byzantina* is used commonly to designate the cultivated varieties derived from *Avena sterilis*, and that it has been used in this sense since 1848, when Koch (*63, p. 392*) published the first description of this species.

Trabut has made detailed botanical studies of red oats and has published a number of papers (*131, 132, 133, 134,* and *135*), one of which has been translated into English (*135*). He holds that *Avena sterilis* has given rise to oats adapted to the warm countries and to saline soils, and *Avena barbata* has given rise to races adapted to dry countries. Experiments in the United States have shown the Burt oat to be adapted both to southern conditions and dry climates. Trabut believes that *Avena sterilis byzantina*, as he here names it, has largely lost the characters of the *sterilis* type and constitutes the last stage before reaching the cultivated form. With regard to it he wrote as follows:

> In 1907 M. Hackel wrote me that he considered this form as intermediate between *Avena sativa* and *Avena sterilis* and named it provisionally *Avena sativa biaristata*.

Hitchcock (*52, p. 110-113*), who describes oat species found in North America, states, apparently on the authority of Trabut (*135*) and Norton (*99*):

> The Algerian oat grown in North Africa and Italy and the red oat of our Southern States are derived from *A. sterilis*. A few varieties adapted to dry countries are derived from *A. barbata*.

Warburton (*143*) points out that—

> the theory that all our cultivated varieties of oats have not been derived from *Avena fatua*, but that certain forms adapted to warm climates have been developed from *A. sterilis*, is not, however, entirely new.

He cites Norton (*99*) as having previously held that idea and takes exception to the following editorial statement which precedes the English translation (*135*) of Trabut's article:

> The prevailing belief that oats can not be grown in the southern United States is probably based on the fact that all the experiments made there have been with cold-climate oats. A great deal of money has already been lost by such attempts, foredoomed to failure because of unsuitableness of the material, although suitable material might have been had, and the country's wealth thus enormously increased had growers studied the genetic history of the cultivated oats earlier.

Warburton apparently believes these statements to be misleading and er-

neous because he states that oat rowing in the South is a profitable arm enterprise and oat experiments onducted in the South have not been ltogether with varieties belonging to *vena sativa*, the northern oat group. He further states that the Algerian ats discussed by Trabut usually have een discarded from experiments in he South, as they appear to be inferior o the Red Rustproof both in yield nd winter hardiness.

In a very complete paper on the general subject of the immunity of plants from infectious diseases Vavilov (*139*) describes *A. byzantina* as being absolutely immune from the oat smut, *Ustilago avenae*, highly or completely resistant to the attacks of crown rust, *Puccinia coronifera*, and susceptible to stem rust of oats, *Puccinia graminis*. He also states that this oat is geographically and phylogenetically distinct from ordinary European susceptible oats. If the variety thus described by Vavilov is the same as or similar to the Burt variety as known in the United States, his work confirms, in a general way, the opinions of American investigators on the disease resistance and distinct taxonomic position of this variety. In an earlier paper Vavilov (*138*) states his belief that *A. fatua*, *A. sterilis*, and *A. ludoviciana* may be regarded as the ancestors of our cultivated oats. He suggests the use of physiological tests in genetics and systematics. The cytological data of Kihara (*61*) and Nikolaewa (*88*) suggest cytology as a further method for use in classifying oat species.

Because of the apparent agreement of many features mentioned by these earlier writers as being characteristic of *Avena byzantina* with the characters of the cultivated varieties of red oats grown in the United States, it seems proper to propose that the name *Avena byzantina* be applied to the cultivated varieties of red oats in the South, in place of the name *Avena sterilis*, now used by nearly all American botanists and agronomists.

It will be recalled that a distinct Latin name, *Avena sativa*, is applied to the cultivated varieties derived from *Avena fatua*, while the cultivated forms derived from *Avena sterilis*, though differing just as widely from the wild *sterilis* as the varieties of *Avena sativa* differ from the wild *fatua*, are designated by the same Latin name as the wild species. It would seem logical to follow the usage of the European workers and apply the name *Avena byzantina* to the red oats grown in the southern United States.

This name would seem especially suitable when applied to such varieties as Burt and Fulghum, which in many respects are intermediate between *Avena sativa* and the red-oat group, until now designated as *Avena sterilis*. The writers have observed some forms of Burt kernels which very closely resemble those of *A. sterilis byzantina*, as illustrated by Trabut (*135*). The name *Avena byzantina* has been used in this sense by Trabut (*135*), and by Schulz (*119*), who states that many forms of this species closely resemble *Avena sterilis*, while other forms can scarcely be distinguished from *Avena sativa*. Schulz (*120*) considers the group of cultivated varieties designated as *Avena byzantina* to be polymorphous, a condition certainly characteristic of the Burt oat, as described in the present paper. Salmon and Parker (*117*) have suggested that the Fulghum oat may be of hybrid origin, the result of natural crossing between cultivated forms of *Avena sativa* and *Avena sterilis*, thus adding strength to the argument for the name *Avena byzantina*, as used in Europe.

As mentioned by Schulz (*119*, *120*), the *Avena algeriensis* of Trabut is considered synonymous with *Avena byzantina*, which would stand on the basis of priority.

Schafer, Gaines, and Barbee (*118*), in grouping Washington oat varieties, state:

> The specific name for cultivated red oats is changed to "*byzantina*" in this bulletin to distinguish them from the wild red oats, *Avena sterilis* L. *Avena byzantina* C. Koch is used in order to have a term to conform with *Avena sativa* L. as *A. sterilis* L. conforms with *A. fatua* L.

The name *Avena byzantina* was used by the above authors in response to a statement from T. R. Stanton, agronomist in charge of oat investigations, that the Office of Cereal Investigations was making this change in nomenclature on the basis of the information presented in the above literature review. The term *Avena byzantina* C. Koch (*A. sterilis* L.) was used in the Yearbook of the Department of Agriculture for 1922.[10]

[10] BALL, C. R., and others. OATS, BARLEY, RYE, RICE, GRAIN, SORGHUMS, SEED FLAX, AND BUCKWHEAT. U. S. Dept. Agr. Yearbook 1922: 469–568. 1923.

INHERITANCE OF SPIKELET CHARACTERS

In reviewing the literature on the inheritance of particular characters in oats it has been thought best to summarize the results under the heads of the various characters studied in the Burt oat. These were (1) spikelet disarticulation, (2) floret disjunction, (3) basal hairs, (4) awns, and (5) lemma color.

SPIKELET DISARTICULATION

At maturity or in threshing the oat spikelet becomes detached from the pedicel on which it is borne. (Its morphology is discussed more fully later in this paper.) In different species and varieties this disarticulation of the spikelet occurs in somewhat different ways, which result in very different appearances of the tissues at the base of the lower floret or kernel.

According to Etheridge (*33*) the basal form of the oat grain has been little used in classifying oat varieties. He states that the callus, the somewhat swollen tissue at the base of the lemma, is an insignificant part of the oat grain. There is sufficient reason for believing that the character of basal form in the oat kernel is of more importance than his statement would indicate. The terms "sucker-mouth," horseshoe base, basal scar, cicatrice, and others have been used by various writers to describe the thickened indurated tissue at the base of the kernel which here is termed the callus.

Surface (*127*), Love and Craig (*70*), and Fraser (*37*) have studied the inheritance of the form of the base. The results obtained by all of these investigators indicate the dominance of the absence of basal scar, characteristic of *Avena sativa* varieties, over its presence, as exhibited by *Avena fatua*, *Avena sterilis*, and the cultivated varieties of the latter which here are grouped as *Avena byzantina*.

The writers have found this character to be of considerable interest, as in its genetic behavior it is one of the most constant of the kernel characters studied.

Etheridge (*33*) cites Denaiffe and Sirodot (*26*) as having characterized various forms of the base according to the obliquity of the scar produced by the detachment of the lower floret from its pedicel, but these authors do not relate the form of the base to the more definite character of articulation or nonarticulation. Böhmer (*9*) and (*10*) also mentions several forms of the base of the lower floret, but does not use them in his classification. Trabut (*135*) makes but little use of the basal form in his paper on the Origin of Cultivated Oats.

Surface (*127*) reports the results obtained from crosses between *Avena fatua* and *Avena sativa*, variety Kherson. He states that the wild form showed an expanded oval ring at the base of the lower floret and that in the cross with Kherson the basal form showing no scar (*sativa*) was dominant, or nearly so. He found the following characters correlated with the "wild" type of base on the lower grain: (1) "Wild" base on the upper grain; (2) very heavy awns on the lower grain of every spikelet; (3) very heavy awns on the upper grain of every spikelet; (4) heavy pubescence on the pedicel of the lower grain; (5) heavy pubescence on the pedicel of the upper grain; (6) heavy pubescence on all sides of the base of the lower grain; (7) heavy pubescence on all sides of the base of the upper grain.

Surface (*127*) detected exceptions to the linkage, or crossovers, between the factors for pubescent glumes and black glumes (about 0.7 per cent) and the inhibitor of glume pubescence and the factor for "cultivated" base (about 1.5 per cent). Bartlett (*8*) has reviewed the work of Surface, above cited, on linkage and crossing over in oats. He believes that some of the cases considered as linkage by Surface (*127*) can be interpreted in a simpler manner. For instance, if the first floret is awned, it is a fair assumption that the second is potentially the same, even though its position be such that manifestation of the character is a physiological impossibility. He suggests that a factor linked with the factor for cultivated base may be conceived as a partial inhibitor of the pubescence factor.

Wilds[11] found, in crosses between *Avena fatua* and cultivated varieties of *Avena sativa*, that the character of the base is determined by a single pair of genes, the *sativa* type being dominant. The *fatua* base showed perfect linkage with the factors for strong awns and dense basal pubescence.

Love and Craig (*70*), in crossing *Avena fatua* with *Avena sativa*, variety Sixty-Day, found that in the segregation of the F_2 generation the sativa-like form of base is dominant or partially so.

[11] WILDS, G. J. INHERITANCE OF GLUME CHARACTERS IN AVENA. 1917. [Unpublished thesis, Cornell Univ., Dept. Plant Breeding.]

ratio approximating 1:3 was obtain-l, the wild form being recessive. hey state that the same has held true n a large number of other crosses in hich the "wild" type has been used. n studying the segregation in the third nd fourth generations they found no ellow oat exhibiting the "wild" base. These results agree with those of urface (*127*), as would be expected, or Warburton and Stanton (*146*) elieve that the *sativa* parents, Kherson nd Sixty-Day, used by these different nvestigators are practically identical. t is known, however, that strains xist within each of these varieties hich differ in kernel color and other haracters. For instance, three pure-ine selections from Kherson, namely, Albion (Iowa No. 103), Iowar, and Nebraska No. 21, all have white-glumed kernels.

Wiggans [12] studied the basal connection in the cross Red Texas × Swedish Select. In F_1 there was an articulation of the outer floret, but this was not so pronounced as in the Red Texas parent. All types were found in F_2, ranging from the Red Texas to the Swedish Select type. The plants were classified as having Red Texas base, intermediate, and Swedish Select base, giving a close fit to a 1:2:1 ratio. These data indicate that there is only one pair of factors involved in determining the basal connection in this cross. There was evidence of linkage between the Red Texas type of base and the Red Texas rachilla character.

Fraser (*37*), in crossing Burt and Sixty-Day, found the F_1 basal form to be intermediate. In F_2 a ratio of three Sixty-Day to one Burt type of base was obtained. His results pointed to the existence of linkage between the Burt type of base and the fully awned condition. There also was evidence of linkage between the Burt base type and the presence of medium long basal hairs. Fraser stated that 4.14 per cent of crossovers occurred between awns and base type and that there was 1.79 per cent of crossing over between base type and basal hairs. He states that the strain of Burt used as parental material in these crosses had a dense covering of mid-length hairs on the sides of the basal callus and that the lower kernel had an articulation much like that of *Avena sterilis*. The Sixty-Day parent had the base and articulation characteristic of *Avena sativa*. Basal hairs seldom were present.

FLORET DISJUNCTION

The florets of an oat spikelet are connected by the clavate segments of the rachilla. The articulation at the juncture of two segments is not very evident, owing to the structure of the spikelet. The base of the lemma of each floret is attached to the enlarged apex of the segment which bears it and entirely surrounds the slender base of the segment which bears the next higher floret. This latter segment therefore appears to grow out from the basal tissue of the inrolled lemma next below.

When florets of *A. byzantina*, the cultivated red oats derived from *A. sterilis*, are separated the rachilla segments bearing the second and third florets usually remain attached to the bases of those florets. When florets of *A. sativa*, the cultivated oats derived from *A. fatua*, are separated the bearing segments usually disarticulate from the base of the floret and remain attached to the face of the next lower floret.

The attachment of the rachilla segment to the base of the second and subsequent florets in *A. byzantina* is very firm. In threshing the florets of the spikelet often remain attached together. When they are forcibly separated the segment breaks near its base, as pointed out above. In *A. sativa*, on the other hand, the separation by disarticulation at the base of the floret takes place very readily.

Norton (*99*) apparently was the first American investigator to call attention to the firm union of the first and second florets with their connecting rachilla segment in the cultivated varieties derived from *Avena sterilis*.

Trabut (*135*) made use of this character in his classification of oats. He states, however, with regard to *Avena barbata* that—

> there is little difficulty in finding all the characters of *Avena barbata*, not taking into account the functioning of the articulation and the pilosity, two fluctuating characters without value, as we have seen in *Avena sterilis* and *Avena fatua*.

Etheridge (*33*) states that the specific character of connection of the kernels has not been used extensively before in the classification of cultivated varieties of oats. He found this character to be of great value for distinguishing the cultivated forms of *Avena sterilis* from those of *Avena fatua*. In his review of the literature on oat classification he refers to Denaiffe and Sirodot (*26*) and to Broili (*12*) as using the form and hairiness of the rachilla

[12] WIGGANS, R. G. THE INHERITANCE OF CERTAIN CHARACTERS IN A CROSS BETWEEN RED TEXAS AND SWEDISH SELECT OATS. 1918. [Unpublished thesis, Cornell Univ., Dept. Plant Breeding.]

as distinguishing characters in their classifications of oats. Schulz also is cited as having used the character of the rachilla connection to differentiate *Avena sterilis* from *Avena fatua, Avena barbata,* and *Avena wiestii.*

Von Tschermak (*137*) describes the linkage groups observed in crosses between cultivated and wild oats. There is absolute coupling between brittleness, or the falling apart of the spikelet on ripening, and complete beardedness. There is complete incompatibility of stiff hairs on the lemmas, as in the wild oat, and the yellow color of the cultivated oat. Brown floret color, as in the wild oat, and the glabrousness of the cultivated oat also are incompatible.

Wiggans [13] studied the breeding behavior of the rachilla character in a cross between Red Texas and Swedish Select oats. The rachilla disjunction of the F_1 kernels was very similar to that of the Red Texas parent, each rachilla segment remaining attached to the base of its floret when threshed. This character is more definite than the basal connection, but in F_2 all gradations from the typical Red Texas disjunction to the typical Swedish Select were found. The Red Texas type of disjunction was dominant, and it seems fairly certain that the kind of disjunction is determined by one pair of factors.

Fraser (*37*) has reported a cross of Burt and Sixty-Day in which he found that the "non-Burt" type of disjunction was dominant to the "Burt" type. He states that the Burt type is similar to that of *Avena sterilis.* He found that three non-Burt plants with the disjunction appeared in F_2 to one with the Burt form. Fraser also found linkage between the Burt disjunction type and the fully awned condition, 4.14 per cent of crossovers being found in 2,341 individuals. He states that the Burt oat used as parental material had the *A. sterilis* disjunction type, while the Sixty-Day had the usual *A. sativa* characters in all respects.

BASAL HAIRS

Basal pubescence or the hairs borne on the callus at the base of the oat floret has been given varying amounts of attention by different investigators.

Nilsson-Ehle (*90*) observed that certain oat varieties had long hairs on the callus, others had short hairs, while still other forms had few or no hairs. When a long-haired form was crossed with a short-haired form, the resulting recombination produced some individuals having no basal hairs and others having stouter hairs than either parent. The hairs of the rachilla were found to be inherited quite independently of the basal hairs.

Zade (*153*), in discussing the results obtained from a cross of *Avena fatua* by *Avena sativa,* states that F_1 was intermediate and that the F_2 showed the two parental and the intermediate types of basal hairs. The progeny segregated in a 1 : 2 : 1 ratio.

Trabut (*135*) makes use of the character of basal hairs in tracing the origin of cultivated forms from the wild oat species.

Etheridge (*33*) in reviewing the work of previous systematists states that the basal hairs frequently are employed by botanists, notably Hitchcock (*52*) and Britton and Brown (*11*), in characterizing Avena species. He states that Denaiffe and Sirodot (*26*) are the only authors who have made definite use of the basal hairs in classifying cultivated forms of *Avena,* although Böhmer (*10*), Broili (*12*), and Fruwirth (*38*) mentioned this character in discussing the morphology of the oat floret and distinguished types of basal hairs on the basis of differences in their form and frequency. In his classification of oats, Etheridge uses a modification of the systems of Broili and Fruwirth. Fischer (*35*), according to Etheridge, holds that the hairs on the base of the oat kernel are a mark of degeneration, and that they occur more often in the winter varieties than in others.

Surface (*127*), reporting on the cross *Avena fatua* × *Avena sativa,* variety Kherson, states that in F_1 basal pubescence was present and the base form was intermediate but more closely resembled that of the *sativa* parent. With regard to the F_1 of this cross he states that the lower floret is pubescent on the back but the upper is entirely glabrous. There is a fairly heavy tuft of hair at the sides of the base of the lower floret but none on the upper. Surface also found that there was correlation between the "wild" base and heavy pubescence on all sides of the upper floret. He also found that pubescence on all sides of the lower floret of the spikelet was correlated with the "wild" type of base.

Fruwirth (*39*) found that the basal hairs on the oat kernel are rather constant in breeding behavior, and that selections for nine years did not effect any change in the density or length of the basal hairs.

[13] WIGGANS, R. G. OP CIT.

Wilds [14] studied the inheritance of basal hairs in crosses of *Avena fatua* with cultivated varieties of *Avena sativa*. He found that the length of the basal hairs was determined by a single pair of genes, shortness being dominant. These genes segregated independently of the other kernel characters studied.

In addition to basal hairs, the kernels of the wild oat parent of these crosses have dorsal hairs. The factor which determines the presence of hairs on both the lower and upper florets is linked with the black color factors.

Love and Craig (*70*) crossed *Avena fatua* with *Avena sativa* variety Kherson. Their results were similar in many ways to those of Nilsson-Ehle (*91*) and Surface (*127*). They observed the intermediate F_1 type with basal hairs on either side but not on the back of the kernel. In F_2 they found the sativalike base dominant, or at least partially so, to the type of base of the wild oat. They also found some linkage between the wild type of base of the wild oat and pubescence, and that the yellow form appeared to carry an inhibitor for pubescence.

Wiggans [15] observed the behavior of the basal hairs in the cross Red Texas × Swedish Select. The F_1 kernels had only a few basal hairs which were approximately the same length as the basal hairs of the Red Texas parent. The F_2 plants were grouped into two classes, hairy and glabrous, although within the first group there was a wide variation in the number of hairs. There was not a great amount of variation in the length of hairs, although a few individuals appeared with much shorter hairs than the Red Texas parent.

The results obtained in F_2 and F_3 may be explained on a two-factor hypothesis.

Fraser (*37*), in describing the Burt × Sixty-Day cross, states that a strong linkage exists between the fully awned condition and medium long hairs at the base of the grain. In 2,341 individuals there was about 5 per cent of crossovers. He found short basal hairs or no basal hairs to be dominant to medium long basal hairs. In F_2 he obtained a ratio of three of the former to one of the latter.

AWNS

The awn is an extension of the midrib of the lemma and usually arises at a point slightly above the middle of the dorsal surface. In most wild species of oats the lemmas of all the florets are awned, the awns being stout and long with the lower portion twisted in a dextrorse or clockwise direction and the upper portion bent over. In all cultivated varieties of *Avena sativa* the awn usually occurs only on the lower lemma, if at all, and usually is small in size. In some varieties derived from *A. byzantina* awns occur occasionally on both florets and sometimes they are twisted or geniculate.

Raum (*108*) considered the presence of awns a very important character in oats, although influenced by climatic conditions and particularly by the rainfall. According to this investigator the weight of the floret and also of the lemma and of the caryopsis increased approximately 10 per cent with the presence of the awn. Completely awnless varieties of oats, however, were not found.

Norton (*99*) found that awned and awnless oats when crossed gave a partially awned type in F_1, which in F_2 split up in a ratio of 1 awned to 2 partially awned to 1 awnless.

Fernekess (*34*) observed that the presence of awns was associated with heavier kernel weight in oats.

Zade (*153*), on crossing a cultivated variety of *Avena sativa* with *Avena fatua*, found in F_1 that the lower floret of the spikelet was awned, which showed presence of the strong awn to be dominant, to some extent at least, over its absence. In the F_2 he observed a ratio of 1 awned to 2 partially awned to 1 awnless.

Nilsson-Ehle (*95*) observed in a cross between a black, strongly awned and a white awnless oat that the F_1 showed the awn on the lower floret of the spikelet. In F_2 the ratio obtained was 1 awned to 2 partially awned to 1 awnless. He found awns to be produced more commonly by black and by white kernels than by yellow ones. He attributed the failure of yellow kernels to produce awns to an inhibitor carried by kernels of that color. He also observed that environmental conditions greatly influence the production of awns in cultivated varieties. He stated that plants having the gene for producing awns may fail to show the awns because of environmental conditions.

Trabut (*135*) in tracing a series of forms between the wild *Avena sterilis* and its cultivated derivatives observed a gradual reduction in the number of awns per spikelet and in the strength of the awns.

[14] WILDS, G. J. OP. CIT.

[15] WIGGANS, R. G. OP. CIT.

Etheridge (*33*) in his classification of oat varieties made use of the awn as a character of secondary importance. He states that the geniculate awns appear abundantly only in a few half-wild varieties, but in such cases they are recognized as a distinguishing character. It is not believed by the present writers that this statement of Etheridge is fully justified, as some of our most valuable cultivated varieties, such as Swedish Select, normally have this type of awn.

Etheridge (*33*) found that in respect to presence or absence of awns, together with their form, the varieties under study have remained constant. In his review of the work of different authors he states that Körnicke and Werner (*64*) made the primary division of the principal groups of oats according to the number of awns per spikelet, that Denaiffe and Sirodot (*26*) used the awn as a distinction between varieties, that Atterberg (*5*), Nilsson (*89*), and Böhmer (*10*) made no use of the awn, and that Broili (*12*) believed the awn to be of little or no value in classifying oat varieties.

Surface (*127*), in crossing *Avena fatua* with *Avena sativa*, variety Kherson, found the F_1 to bear awns on only the lower floret and that the second floret of the spikelet was never awned. In F_2 he found stout awns on the lower floret and awns on the upper floret, both correlated with the *fatua* type of base. He found a slightly greater proportion of the yellow-kerneled plants than of the other colors which were awnless but did not consider the difference significant. According to Surface there did not seem to be any marked correlation between yellow color and absence of awn in this cross.

Love and Fraser (*69*) made a cross between Burt, a weak-awned [16] variety, and an awnless strain of Sixty-Day and observed that the F_1 plants were almost awnless. In F_2 a ratio of approximately 2 awned to 1 awnless plant was found. In F_3 the fully awned forms bred as pure recessives, the partially awned plants bred in the ratio of 3 awnless to 1 awned, and certain awnless F_2 plants apparently were heterozygous as they produced awns in F_3. Love and Fraser do not believe the yellow color in Burt carries the inhibitor for awns as does the yellow color in Kherson. According to these authors it appears that the yellow color in Sixty-Day carries an inhibitor for awn development.

In the cross Red Texas × Sixty-Day the same authors found only 1.3 per cent of awns in F_1. The awnlessness of the Sixty-Day parent apparently was dominant in F_1 over the weak-awned type. In F_2 an approximation of the 1: 2: 1 ratio was observed. The fully awned type was found to breed as a pure recessive, partially awned types were shown to be heterozygous, and awnless F_2 plants broke up in F_3, showing that not all awnless F_2 plants can be considered as pure dominants. Love and Fraser state that weak awns and awnlessness in oats probably are due to a 1-factor difference. Sixty-Day may carry an inhibitor for awn development linked with the yellow color, but Burt evidently does not carry such an inhibitor, as awned yellow Burt types are found in numbers.

In a cross of the strong-awned *Avena fatua* with the awnless Sixty-Day the same authors found F_1 to be intermediate. In F_2, 133 awnless, 215 intermediate, and 112 plants exhibiting the "wild" type were found. These authors state that in the Burt variety, which they consider a *sterilis* type, the fully awned condition was correlated with the Burt type of disjunction and midlength basal hairs. They also state that 2-awned spikelets are found only in fully awned panicles.

Wilds [17] studied the inheritance of awn type in crosses of *Avena fatua* with Tartar King and Sixty-Day. The strong awn was found recessive to the awn of intermediate type (awnless) and partially awned plants were considered as dominants.

Love and Craig (*70*) reported that, in a cross of *Avena fatua* and *Avena sativa*, variety Sixty-Day, the F_1 was intermediate and that there was some relation between the yellow color and the absence of awns. In another paper (*71*) they state that for the "weak" awn the fully awned condition is recessive and the character seems to occur in a simple 1: 2: 1 ratio.

Meunissier (*77*) considers that the awn is a fluctuating character. He states that there are strains of oats mostly much awned, others which have very few awns, and still others which are entirely without awns. Absence of the awn appears to be clearly a recessive character. He states that the twisting of the awn is sinistrorse (in a spiral to the left).

[16] The "weak" awn class of Love and Fraser includes the "nontwisted" awn class described in the present paper.

[17] WILDS, G. J. OP. CIT.

Wiggans[18] studied the behavior of he awn character in the cross Red Texas × Swedish Select. The F_1 spikelets possessed only one awn and his was slightly twisted and bent, a ypical intermediate condition. The F_2 plants were classified in regard to wns per spikelet as (1) one strong wn (2) one intermediate awn (3) ne weak awn, and (4) two weak awns. The last two classes proved to be the same in their breeding behavior and should be considered as one class. If this is done a close approximation to the following ratio is obtained: 1 (one strong awn) : 2 (one intermediate awn) : 1 (one or two weak awns). These results indicate that one pair of factors determine the kind of awns in this particular cross, although both parents contain at least one other factor for awning.

It was found that yellow plants practically always had either one weak awn or two weak awns on each spikelet and that the plants classified as whites generally had only one awn to the spikelet and this one was strong in the majority of cases. There are indications of a strong linkage between the yellow color and the weak-awned condition. The linkage between white color and strong-awned spikelets was not so strong as that between yellow color and weak awns but is significant.

In the cross Burt × Sixty-Day Fraser (*37*) found nearly complete dominance of the awnless condition. According to Fraser both parents carry the factor for awning, but this factor was prevented from operating in Sixty-Day by an inhibitor linked with yellow color. In the first generation the production of awned plants is dependent on the extent of dominance which this inhibitor displays, which in turn probably is dependent on environmental factors. In F_2, awnless, partly awned, and fully awned plants are produced approximately in the ratio of 1 : 2 : 1, the ratio of plants not fully awned to plants fully awned being close to 3 : 1. The fully awned plants when tested in F_3 proved to be pure recessives. Nearly all partly awned plants were heterozygous and gave ratios of approximately 3 plants not fully awned to 1 fully awned. The awnless F_2 plants were of two kinds, those breeding true, or practically so, and those which segregated in a manner similar to an F_1 plant.

Zhegalov (*160*) has reported the results of a number of species crossed in oats and discusses the inheritance of awns and other characters in these crosses.

Marquand (*75*) found that the percentage of awned spikelets in panicles taken from unselected varieties is constant on the whole, and unaffected by external conditions though probably dependent upon multiple factors.

LEMMA COLOR

In this review it is understood that all references made by the various authors to color of glumes, kernels, and grain were intended to apply to the floret and more definitely to the lemma or flowering glumes.

Körnicke and Werner (*64*) in their classification of oats used lemma color to distinguish the main groups of varieties of *Avena sativa* and *Avena orientalis*.

Von Tschermak (*136*) found that black color in the glumes was dominant over the light or yellowish color.

Wilson (*151*) crossed Black Tartarian oats with Goldfinder, a white oat. The F_1 plants had rich brown grains. In F_2, black, brown, yellow, and white kernels could be distinguished. By including the blacks and browns in a dark-kerneled class and the yellows and whites in a light-kerneled class, a ratio of 2.99 of the former to 1 of the latter was obtained. Other crosses between Black Tartarian and white-seeded varieties gave results in F_2 which clearly indicated the dominance of dark-kernel color.

Norton (*99*) found that black and white oats when crossed gave a brown hybrid in F_1, but in F_2 they produced a ratio of 1 black: 2 brown: 1 white. In succeeding generations the extracted black and the extracted white types bred true.

Roberts and Freeman (*114*) studied an apparent genetic alteration of the color type of Red Texas oats from red to black under Kansas conditions but found that close-pollinated plants of each sort yielded seed which came true, indicating no sporting of one variation from the other.

Nilsson-Ehle (*91*) noted a slight variation in the lemma colors of oats as the result of environment, although he believed that in the main they tend to breed true. He found the range of variation in dark-colored florets to be from black to brown or brown to black. Crosses of the following types were made: Black × white, yellow × white, gray × white, black × yellow. In crossing black and white oats he found

[18] WIGGANS, R. G. OP. CIT.

that black was dominant over white and that there were two factors for dark color. The presence of one of these factors produced gray, while when both were present the kernel was black. In the absence of both color factors white kernels resulted.

Nilsson-Ehle (*92*) described cases of the spontaneous omission of the color factor in oats, giving rise to white and gray kernel types in pure-line material of black varieties.

Zade (*154*) in his studies of inheritance in *Avena fatua* observed that the kernel colors tended to breed true.

Thatcher (*129*) found as the result of experiments in crossing oats that black hull was dominant over white hull.

Nilsson-Ehle (*95*) showed that there is a very definite linkage of the factor which inhibits awning and the factor for yellow color.

Etheridge (*33*) states that the color of the lemma when ripe has been accorded various degrees of importance in classification by other investigators. In some cases Etheridge used color as the basis for separation of the principal groups. He states that it is the most conspicuous character of the oat grain, that it is certainly inherited, and therefore is of particular use in identification and description. He points out that while the color is affected by changes in environment and may pass into different tones of the same general hue, it does not transgress the limits of the type. One must not attempt to make fine subdivisions of color, for the distinctions may be lost by variation within the type. In his review of the work of previous investigators Etheridge states that Nilsson (*89*) Denaiffe and Sirodot (*26*), and Dufour and Dassonville (*27*) all used color in making varietal distinctions. He also states that Böhmer (*9*) used color as a final means of distinguishing varieties, while Fruwirth (*38*) believed color of little importance, and Atterberg (*5*) mentions it only as a descriptive character.

Surface (*127*) presents the results obtained from a cross of *Avena fatua* with *Avena sativa*, variety Kherson. He states that the wild parent has a dark brown or almost black lemma color, while the Kherson has a yellow color. He found F_1 to be intermediate in color between the two parents and of a lighter brown color than the wild parent. In F_2 and F_3 it was shown that the results could be explained on the basis of the presence of three separate factors, black, gray, and yellow, each of which is allelomorphic to white.

Gaines (*40*) has studied the inheritance of lemma color in oats in 10 different crosses. The parental combinations used by Gaines in studying this character were as follows: (1) Storm King × Black Tartarian, (2) Regenerated Swedish Select × Black Tartarian, (3) Black (Cereal Investigations No. 290) × Regenerated Swedish Select, (4) Black × Sixty-Day, (5) Black × Palouse Wonder, (6) Black × Hull-less, (7) Sixty-Day × Chinese Hull-less, (8) Black Tartarian × Hull-less, (9) Canadian × Chinese Hull-less, and (10) Storm King × Chinese Hull-less.

In a total of 29,730 F_2 plants a ratio of 24.9 per cent of plants with white florets to 75.1 per cent with black florets was obtained. Gaines believes that the factor for lack of hulls in oats which causes the floral glumes to expand, elongate, and remain widely spread at maturity (the characters that distinguish naked varieties), inhibits the development of the dark color in the floral glumes, for in all the crosses not a single true naked plant developed black floral glumes. If it had not been for the brownish coloring of the palea the naked hybrids which bred like black oats when crossed with white-hulled varieties could not have been distinguished from those that were genetically white.

Love and Fraser (*69*) observed in crosses of Burt with Sixty-Day and with other varieties that the yellow color of Burt apparently carries no inhibitor for awns, but that the yellow color of Sixty-Day apparently carries this inhibitor in a manner similar to Kherson, as described by Surface (*127*).

Wilds [19] studied crosses of the wild oat, *Avena fatua*, with Tartar King and Sixty-Day, and concluded that the genes for color of the flowering glumes segregate independently of each other, each being allelomorphic to white or to its own absence. In the cross of the wild oat × Tartar King (white) the F_2 ratio obtained was very close to the expected 12 blacks and browns : 3 grays : 1 white. In the cross of the wild oat × Sixty-Day (yellow) an F_2 ratio of 12 blacks : 3 grays : 1 yellow was observed.

The ratios obtained in these crosses indicated a negative correlation between yellow lemma color and *fatua* articulation, between yellow color and awns, between yellow color and basal lemma pubescence, and between yellow color and dorsal pubescence.

Caporn (*14*) studied the inheritance of kernel color in crosses between

[19] WILDS, G. J. OP. CIT.

vena nuda, said to be impure as gards color, and the white-husked rieties Thousand Dollar and Ligowa, hich were a mixture of grays and hites. The presence of grays demonrated, however, the dominance of e gray color whenever the cross ay × white actually occurred.

In another cross, Nubian Black × *vena nuda*, the F_1 color was a bright rown sometimes overlaid with a faint rayish flush. Here the F_1 also may ary because the black parent is genetic-ly really made up of at least three dif-rent kinds of blacks, represented ygotically by the formulæ BB B′B′ GG, B B′B′ gg, and BB b′b′ gg, where B nd B′ are factors for blackness and for gray color. In F_2 a ratio of pproximately 3 gray to 1 white was bserved. There was no linkage or epulsion of gray or brown color with ght paleas.

Caporn reports in another paper (*15*) n a cross between Mesdag, a variety ith dark-brown florets and distinct ffinities with *Avena fatua*, and Hope-own, a white-glumed variety. The F_1 florets were somewhat lighter brown han those of Mesdag. No data on he inheritance of color in F_2 are given.

Love and Craig (*70*) present data n a cross of *Avena fatua* and *Avena ativa*, variety Sixty-Day. The result btained in this cross corresponds very losely with that obtained by Surface (*127*). They assumed that *Avena fatua* arries color genes for black, gray, and ellow, and that Sixty-Day has the ene for yellow. They found a strong ndication of an inhibitory factor or actors which prevent not only awn levelopment but also the wild form of emma base and pubescence in combi-nation with yellow color of the lemma.

Love and Craig (*71*) found that in rosses between the Sixty-Day oat and he wild *Avena fatua* the yellow color f the Sixty-Day inhibits the produc-ion of well-developed awns and pubes-ence on the glumes. These crosses lso have shown that different types re found in *A. fatua*. One sort when rossed with the White Tartar King gives 15 plants with lemmas pubescent o 1 nonpubescent in the second gen-ration, while another type produces ratio of 3 pubescent to 1 nonpubes-ent. Two forms of black oats classed s the same variety produced plants vith white florets in the second genera-ion in the ratio of 15 black to 1 onblack.

Meunissier (*77*) states that white lume color is recessive to other colors.

Pridham (*106*) quotes A. E. V. Richardson, of Australia, as having mentioned that in oats all the different tints of kernel color appear to be Mendelian dominants to colorless or white.

Wiggans[20] studied the inheritance of kernel color in the Red Texas × Swedish Select cross. The color of the F_1 florets was red, although a somewhat lighter red than that of the red parent grown under the same conditions. Floret color, as developed under greenhouse conditions, proved to be a difficult character to work with. Four types were evident in F_2, red, gray, white, and yellow, although accurate classification was practically impossible. The data indicate that there is more than one pair of factors which determine color. The factor for red is the dominant one.

Fraser (*37*), in crossing Burt (red) with Sixty-Day (yellow), found F_1 to be intermediate in color. In F_2 difficulties in classification were experienced because of the gradation of colors and the influence of environmental factors. An approximate ratio of 48 reds : 15 yellows : 1 white was observed in F_2. Fraser considers that Burt carries two color factors, red and yellow, while Sixty-Day has only the factor for yellow.

According to Fraser (*37*) the genetic formula for floret color in the Burt variety would be RRYYy′y′. The variety Sixty-Day would have the genetic formula rryyY′Y′. He states that the results in the F_3 generation bear out this theory in a general way. He believed that because of the yellow color factor in the Burt variety which carries no inhibitor for awns, the inhibitory effect of the Sixty-Day factor was obscured. Fraser considered the few brown florets which appeared in the course of his studies to be the result either of mutation or of reversion.

Wakabayashi (*140*) reported a cross between *Avena sterilis*, variety Red Rustproof, and *Avena sativa orientalis*, variety Black Tartarian. He found the black color of Black Tartarian to be a simple Mendelian dominant, and believed that there was some linkage between white color and susceptibility to smut.

PRELIMINARY OBSERVATIONS

Apparently one of the first persons to recognize the variability of the Burt oat was J. B. Norton, formerly engaged in oat breeding in the United States Department of Agriculture. He

[20] WIGGANS, R. G. OP. CIT.

pointed out in 1907 the unusual position of the variety. Wilson G. Shelley, formerly in charge of the experiments of the United States Office of Cereal Investigations at the Akron Field Station, Akron, Colo., observed in 1910 that Burt was a promising early variety and recorded in his field notebook that the kernels of the variety lacked uniformity in color.

In 1916 one of the writers (Parker) observed (*100*) that the Burt oat had potential value because of its resistance to rust. Several strains of Burt were being grown at the Kansas Agricultural Experiment Station when he took charge of the crop improvement project in 1918. A special effort was made to obtain additional strains from all possible sources. Pedigreed selections were made from several strains, and studies of the smut resistance of Burt and other varieties were started. The marked variability of the Burt variety, clearly indicated by the earlier studies of rust resistance, was observed to be very evident with respect to plant and kernel characters.

Preliminary experiments to determine the nature of the variability existing in the Burt oat were initiated by the senior writer at the Akron (Colo.) Field Station in 1919, at the suggestion of T. R. Stanton. These experiments were started with the Burt oat selection, Cereal Investigations No. 293–6–09, made by Wilson G. Shelley when he was at the Akron Field Station. This selection is now named Colburt (C. I No. 2019). The original plan was to use the plant as the unit for study. The kernels were spaced 6 inches apart, 25 kernels to the row, in rows approximately 12 inches apart. Approximately 600 plants were produced from the sowing of 1,000 kernels, and of this number 75 were retained for the study of variation. These plants were threshed by hand in the spring of 1920. General notes were recorded on the plant characters and detailed notes were taken on the floret characters of each plant. The descriptive notes on floret characters may be summarized as follows:

1. In color the florets of some plants were more variable than those of others.
2. The florets from some plants were uniformly dark brown or black, while those from others were uniformly light brown.
3. In some plants the florets were uniformly large, and in many cases the large kernels were very dark in color.
4. The awns borne by the florets of a single plant varied in character. The twisted awn was frequently observed. No plants were found with all of the lower florets of the spikelets bearing awns, although many awnless plants were observed.

No further study was made of this material.

MORPHOLOGY OF THE OAT SPIKELET

A brief discussion of the oat spikelet is given here to make clear the genetic discussion which follows.

The spikelet is borne on the thickened end of the slender, drooping pedicel which terminates the panicle branch. Each spikelet usually contains two or more florets, though one-flowered spikelets occur rarely. No oat varieties are known which produce one, two, or three florets per spikelet, exclusively. The lower two florets usually are perfect, while the third, if present, often is staminate or imperfect. The first floret is the largest and contains the larger kernel or caryopsis.

The two lower glumes, or empty glumes, are somewhat unequal, lanceolate, acute, boat shaped, spreading, glabrous, membranous, and usually persistent. Both usually exceed the lemma or flowering glume in length, except in naked oats.

The rachilla or axis of the spikelet bears all of the florets and connects the spikelet with its supporting pedicel. In some species, as *Avena nuda* and *A. strigosa*, the rachilla segments are elongated and narrowly clavate, while in other species, as *A. sterilis*, *A. fatua*, and many of their cultivated derivatives, the segments usually are shorter and more thickened. Zade (*156*) states that the greatest difference between wild and cultivated oat species is their method of separation from the pedicel.

The floret is composed of the lemma, the palea, and the organs of reproduction, namely, the ovary with its bifid style, plumose stigma, and the three stamens.

The lemma or flowering glume is the lower of the two bracts or scales which form the envelope of the kernel. It is slightly shorter and much firmer in texture than the empty glume. It is ovate-lanceolate or boat shaped, with the scabrous apex bifid or entire. The veins of the lemma and glume appear as slender, riblike striations. In some wild forms the veins of the lemma extend beyond its apex as teeth or awn points and are used as characters in distinguishing species. The number of the nerves is variable, usually ranging from 7 to 11 in cultivated varieties. The base of the

lemma may be extended into a swollen callosity, commonly called the callus.

The dorsal surface of the lemma may be either hairy or glabrous, characters much used in separating oat species. Most wild species of oats are characterized by hairiness of the callus, lemma, and rachilla. The callus, a somewhat swollen, thickened and hardened projection at the base of the lemma, often bears more or less conspicuous bristles, usually termed basal hairs. The presence of these hairs may be observed readily without magnification.

The awn of oats is an extension of the midrib of the lemma, usually arising at a point slightly above the middle of the dorsal surface. In *Avena fatua*, *A. sterilis*, and various other wild species all lemmas are awned. In these the awns usually are stout and long and the basal portion is twisted dextrorsely or in a clockwise direction. That part of the awn above the twisted portion usually is bent or geniculate. In most cultivated varieties derived from *A. fatua* and *A. sterilis* the awn when present occurs only on the lower kernel of the spikelet and then it may be diminutive in size. In some cultivated varieties it occurs only rarely, even on the lower lemma. In varieties of *A. byzantina* awns occasionally occur on both kernels and sometimes they are twisted or geniculate.

Trabut (*135*) observed a gradual reduction in the number and size of the awns in passing from the wild *Avena sterilis* to the cultivated strains represented by *A. byzantina*. Some authors believe the occurrence of numerous twisted and geniculate awns in cultivated oat varieties to be an indication of degeneracy resulting from an unfavorable environment.

The palea or palet, the inner or upper bract or scale of the floret, is a thin parchmentlike scale, the margins of which usually interlock with those of the lemma. The palet may be toothed at the apex. The tightness with which the lemma and palea clasp the caryopsis is an important character in oat classification. In *A. nuda* they inclose the caryopsis rather loosely, while in most hulled varieties the caryopsis is firmly inclosed.

The oat caryopsis is narrowly oblong or spindle shaped, deeply furrowed on the ventral surface, and usually covered with fine hairs, especially at the upper end. The caryopsis usually has no characters of value for classification purposes.

SPIKELET DISARTICULATION

The separation of the lower floret of the oat spikelet from the rachilla or axis of the spikelet is here termed spikelet disarticulation, in contrast to floret disjunction or the separation of the florets of the spikelet from each other. Few experiments have been conducted on the histology of the oat spikelet, and as a result the exact structure of the rachilla is not well understood.

In the wild species, *Avena fatua* and *A. sterilis*, and in most of the cultivated varieties the basal segment of the rachilla usually is short and thickened. Apparently, the basal segment of the rachilla and the projecting basal callus of the lower floret are united obliquely in the lateral plane, the callus being dorsal and the rachilla ventral. In the two wild species named, spikelet disarticulation takes place by means of an oblique abscission layer, apparently located in the cleavage plane between the basal rachilla segment and the callus of the lower floret. It is possible that a true articulation between the base of the lower lemma and the apex of its supporting rachilla segment exists above this abscission layer, and that this abscission layer is formed in the tissue of the rachilla segment itself, but this is very improbable. Separation at this layer in that case would leave a portion of the basal rachilla segment attached to the lower floret.

In the present paper it is assumed that the abscission layer is formed at the base of the callus of the lemma, and that below this abscission layer the tissue is rachilla, while above this layer it is lemma. In *Avena sterilis*, *A. fatua*, and some of their cultivated derivatives the separation at this definite oblique abscission layer leaves a well-defined deep oval cavity, commonly called the scar, or "sucker-mouth," in the face of the callus. A corresponding but shallower depression remains in the face of the disjoined basal segment of the rachilla.

Zade (*156*) states that connection between these two parts, the callus and the rachilla segment, is only at the periphery in the wild species, while in the cultivated oat, *A. sativa*, the central portion also is solid, being filled with a parenchymatous tissue. The writers believe his observations probably are correct for conditions at maturity, but that in fresh, immature plants the union of the rachilla and callus is solid both in wild species and their cultivated derivatives. The cav-

ity apparently is evident only in mature or nearly mature specimens. In many fresh, immature specimens both of wild and cultivated oats the writers have observed no hollow or scar in the callus or the basal segment of the rachilla when these organs are forcibly disjoined.

In the present study the different ways by which spikelet disarticulation takes place were classified as resulting from (1) abscission when the method of spikelet separation was that characteristic of the wild *Avena fatua* or *A. sterilis*, that is, resulting in a pronounced cavity or scar in the base of the lemma; (2) fracture when the method of separation was that most characteristic of the cultivated varieties of *A. sativa*, that is, resulting in a roughened tissue with no observable cavity or scar at the base of the lemma; and (3) semi-abscission when the method of separation was to some extent intermediate between the two, apparently resulting partly from abscission and partly from fracture, and leaving only a slight and often poorly developed cavity or scar in the base of the lemma. These three kinds of disarticulation are shown in Plate 1, A to C.

FLORET DISJUNCTION

The florets of an oat spikelet are connected by the clavate segments of the rachilla. The articulation at the juncture of two segments is not very evident, owing to the structure of the spikelet. The base of the lemma of each floret is attached to the enlarged apex of the rachilla segment which bears it and entirely surrounds the slender base of the segment which bears the next higher floret. This latter segment, therefore, appears to grow out from the basal tissue of the inrolled lemma next below (Pl. 1, D). The manner of separation of the florets from one another, here termed floret disjunction, differs in different species.

The rachilla segments usually are narrowly clavate in shape and variously flattened, rounded, or furrowed. They usually are 1.5 to 3.5 mm. long, except in *Avena nuda*, where they are extremely elongated. In the wild species they often are clothed by stiff hairs. The number and character of these hairs are much used in differentiating between wild and cultivated forms.

In some species, as *Avena fatua* and its derivative *A. sativa*, disjunction of the second and additional florets usually takes place by disarticulation at the apex of the rachilla segment. In such cases the rachilla segment remains attached ventrally at the base of the next lower floret of the spikelet. The attachment of the rachilla segment to the base of the second and subsequent florets is very firm in *A. sterilis* and its derivative *A. byzantina*. The florets of the spikelet often remain attached together during threshing. When forcibly separated, the segment breaks near its base, as pointed out above. But in *A. sativa* the separation by disarticulation at the base of the floret usually takes place very readily.

In the present study it was observed that in some cases the segment breaks across near the middle or even splits irregularly lengthwise.

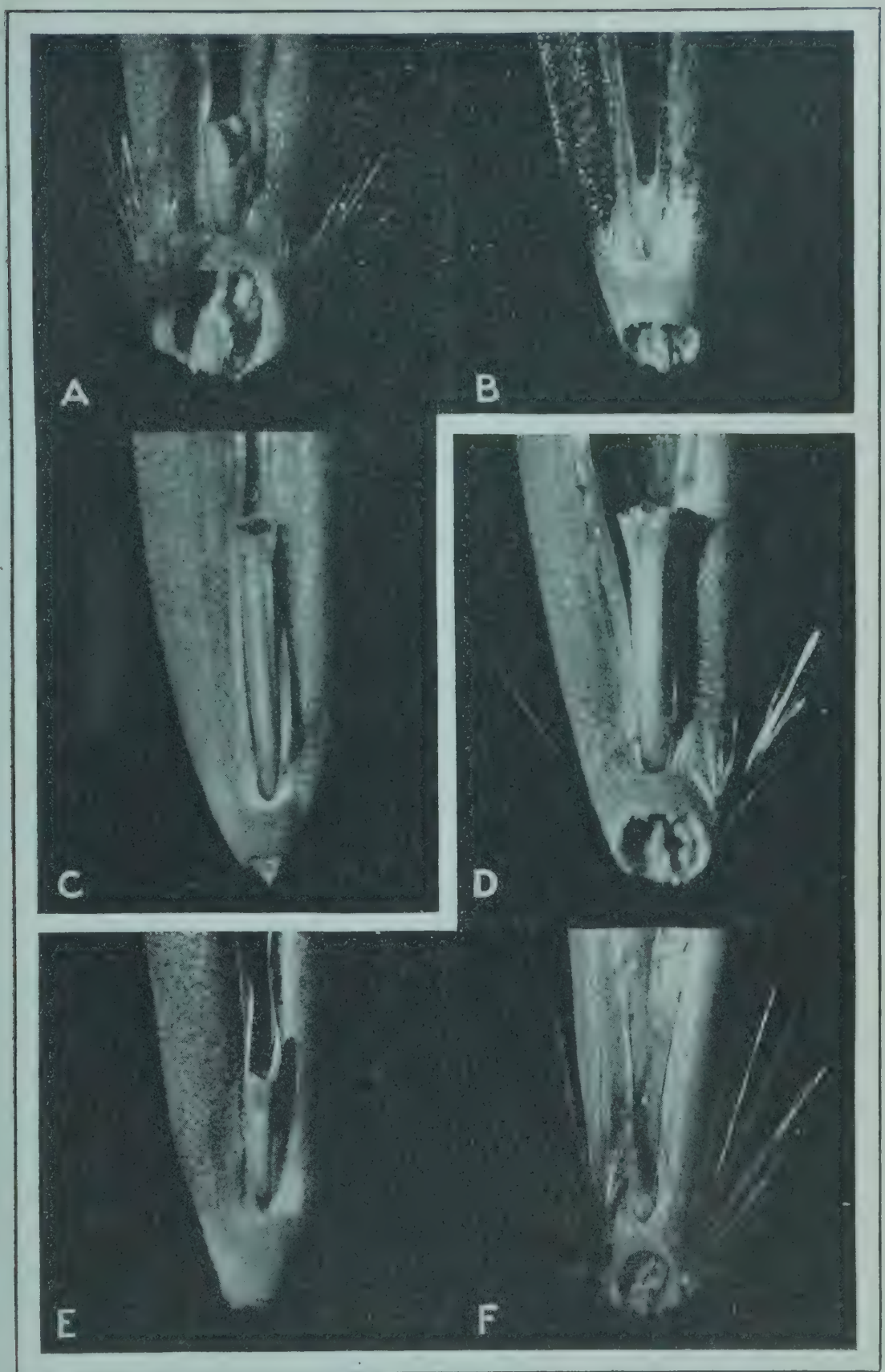
Floret disjunction in this study is described in terms of the nature of the disjunction of the supporting rachilla segment. The term (1) disarticulation is used when the segment disarticulates from its floret, as in *Avena fatua*; (2) the term basifracture when disjunction is by fracture at the base and it remains attached to the floret, as in *A. sterilis*; and (3) the term heterofracture when it fractures irregularly in the middle and the disjunction can not be classified definitely. Plate 1, D to F, shows these three different types of floret disjunction

BASAL HAIRS

The surface of the lemma may be either hairy or glabrous, characters much used in separating oat species. Most wild species of oats are characterized by hairiness of the lemma, callus, and rachilla. The callus often bears more or less conspicuous bristles, usually termed basal hairs. The presence of these hairs may be observed readily without magnification.

The classification of basal hairs in the present paper conforms somewhat to that of Zade (*156*). He classed them as long, intermediate, and short. The writers have discussed them as (1) abundant long, (2) abundant midlength, (3) few, disregarding length, and (4) absent.

The abundant long class of the writers is practically identical with the long class of Zade. The abundant midlength class includes most of those he described as intermediate. In addition, the writers included in this second class a few kernels bearing hairs which though rather short were very abundant. Some of these kernels doubtless would have been put in Zade's short class. The writers' "few" class was in reality a sort of catchall. It included all kernels that were only



A to C.—Spikelet disarticulation, or separation of the lower floret from the basal segment of the rachilla. A.—Disarticulation by abscission, the method occurring commonly in the wild oat species *Avena fatua* and *A. sterilis*, and in some strains of the Burt oat. B.—Disarticulation by semiabscission, more or less intermediate between A and C, apparently resulting partly from abscission and partly from fracture, and leaving only a slight and often poorly developed cavity in the base of the lemma. C.—Disarticulation by fracture. The method of separation most characteristic of the cultivated varieties of *Avena sativa*, resulting in a rough, fractured tissue with no observable cavity at the base of the lemma.

D to F.—Floret disjunction, or separation of the second floret from the first or lower floret. D.—Disjunction by disarticulation. The second floret disjoins by disarticulation of the second rachilla segment from the base of the second floret, the common method in the wild species *Avena fatua* and the cultivated varieties of its derivative, *A. sativa*. E.—Disjunction by heterofracture. The rachilla segment breaks transversely somewhere in the middle portion, or may break both transversely and longitudinally, the latter splitting being very diverse. F.—Disjunction by basifracture. The common method of separation in the wild *Avena sterilis* and the cultivated varieties of its derivative, *A. byzantina*, the rachilla segment breaking at or near its base and remaining firmly attached to the base of the floret.

sparsely hairy whether the hairs were long, midlength, or short. A few kernels with hairs abundant, but so very short that they could not be placed in the abundant midlength class, were also thrown into the "few" class.

Many different classifications of the basal hairs in the oat kernel have been used, and nearly all such classifications have objectionable features. All classifications must be more or less arbitrary, as they are based on number or length of these bristles. A few authors have used a combination of these two characters. Although realized to have objectionable features, the writers believe the following proposed classification might have been followed with profit:

1. Long.
   - Many.
   - Few.
2. Midlength.
   - Many.
   - Few.
3. Short.
   - Many.
   - Few.
4. Absent.

In describing large numbers of oat kernels the writers find all of these classes and subclasses to exist. The different classes of basal hairs used in this study are shown in Plate 2.

AWNS

Various authors have used different terms for designating the different kinds of awns. Here again any classification must of necessity be an arbitrary one and as such is subject to differences of opinion. The terms strong, twisted, geniculate, and weak have been rather loosely used by one author in describing the awn of the oat. Another author has designated different awn conditions by the terms strong, intermediate, weak, and awnless. In describing the material on which data are presented in this paper the terms (1) twisted; (2) nontwisted, long (over 15 mm. in length); (3) nontwisted, short (less than 15 mm. in length); and (4) absent have been used.

The twisted awns usually are associated with the wild forms. These awns often are strongly kneed or geniculate. The percentage of the length of the awn which is twisted varies. In some cases where the twisting is less pronounced, and only one or two twists occur, the bending is too slight to be termed kneed, although bending apparently is very closely associated with twisting. Of the nontwisted awns the long awn may be fully as long as the twisted one but it has no twisting at its base and hence practically always lacks the dark color associated with the twisted base. The nontwisted, short awns are somewhat more variable than the other awn classes discussed. They are always slender, 15 mm. or less in length, grading downward to mere bristlelike appendages, sometimes more like hairs than awns. In kernels having no trace of awns the awn is said to be absent. The various oat awns are shown in Plate 3.

LEMMA COLOR

The oat lemma is of various colors. The principal ones recognized in previous descriptions have been black, gray, red, yellow, and white. In the present classification many kernels which were not dark enough to be called black but too dark to be termed either gray or red are called brown. A few authors previously have recognized brown as a kernel color but generally have considered it analogous to black.

In the present paper the lemmas were classified according to color as follows:

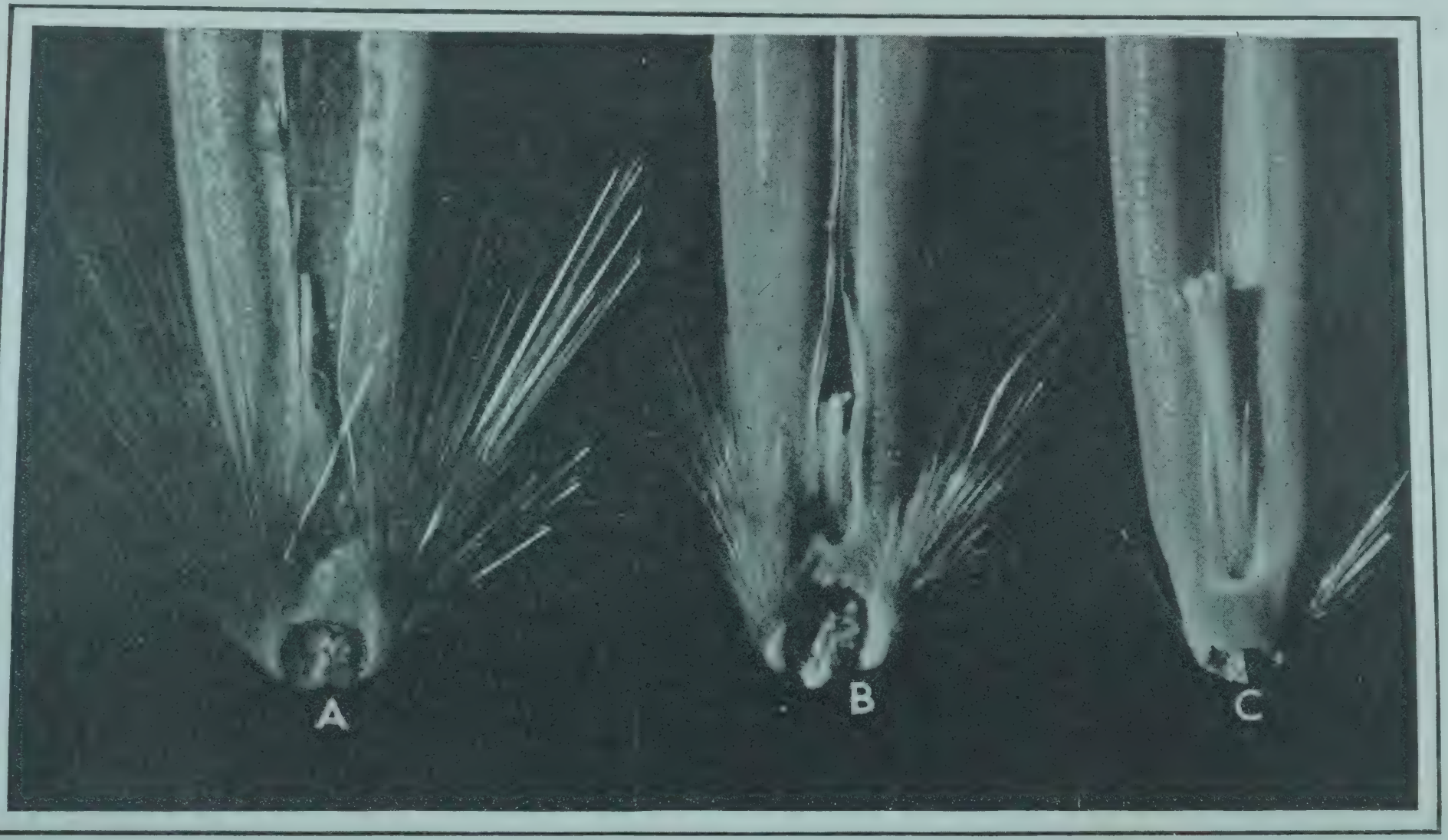
Black, dark brown, light brown, red, variegated, yellow, white. The colors of the lemmas in Burt are shown in Plates 4 to 6, inclusive. The florets with so-called variegated lemmas proved very diverse when their progeny were grown and this color subdivision was abandoned.

EXPERIMENTAL MATERIAL

In 1920 bulk lots of nine different strains of the Burt oat growing in the crop improvement nursery of the agronomy department at the Kansas Agricultural Experiment Station were chosen for the variability studies. This material showed considerable variation not only among the different strains but within each of the strains, and was exceptionally well suited for such a study. The strains used were Kansas Nos. 5020, 5211, 5219, 5022, 6004, 6052, 6076, 6090, and 6094. The accession records on file at the depart-

EXPLANATORY LEGEND FOR PLATE 2

A.—Abundant long basal hairs, characteristic of many wild oats. B.—Abundant midlength basal hairs, a condition somewhat intermediate between wild and cultivated varieties. C.—Few basal hairs. In nearly all cases these hairs are short but rarely kernels are found bearing only a few long hairs.



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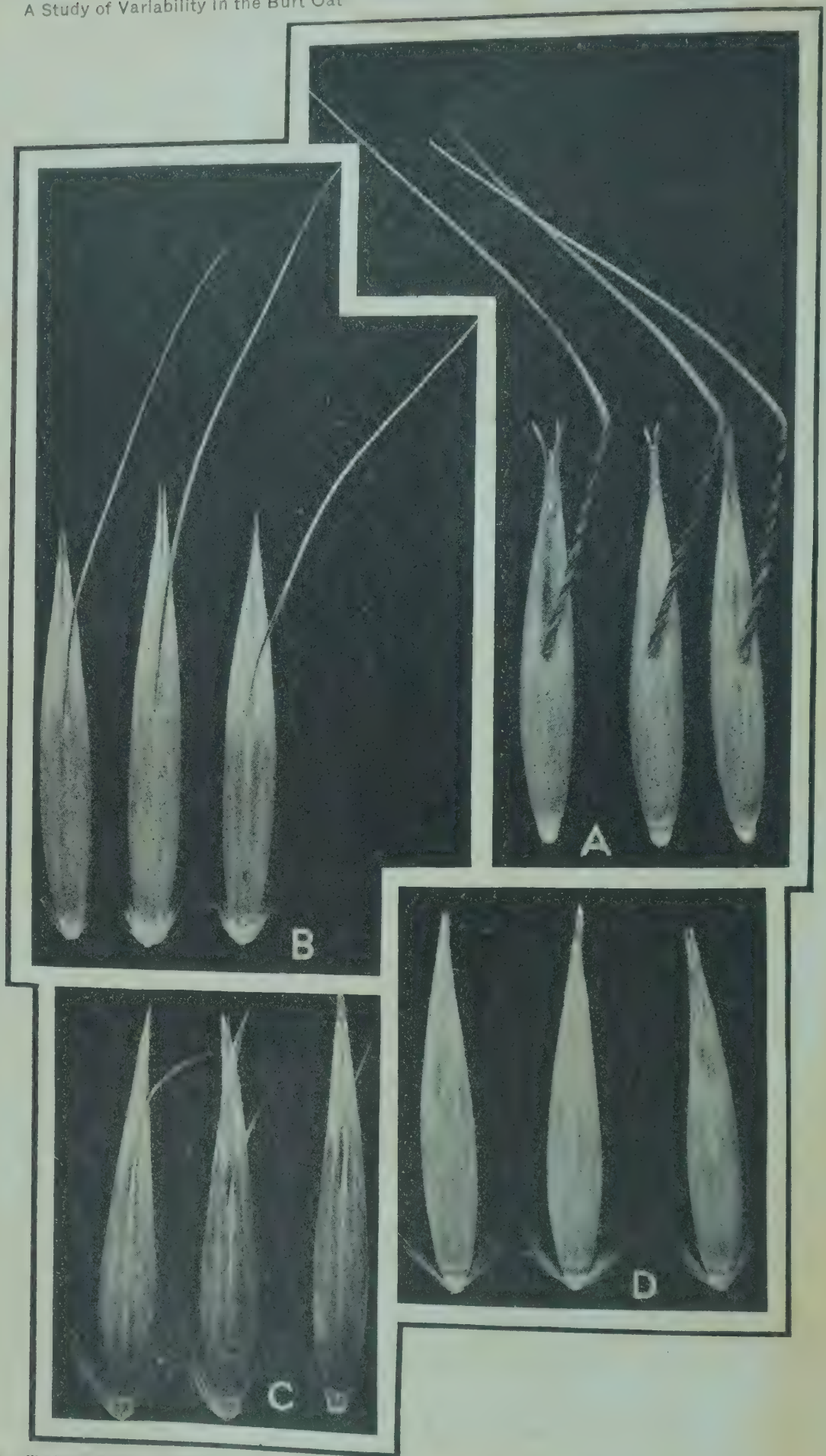
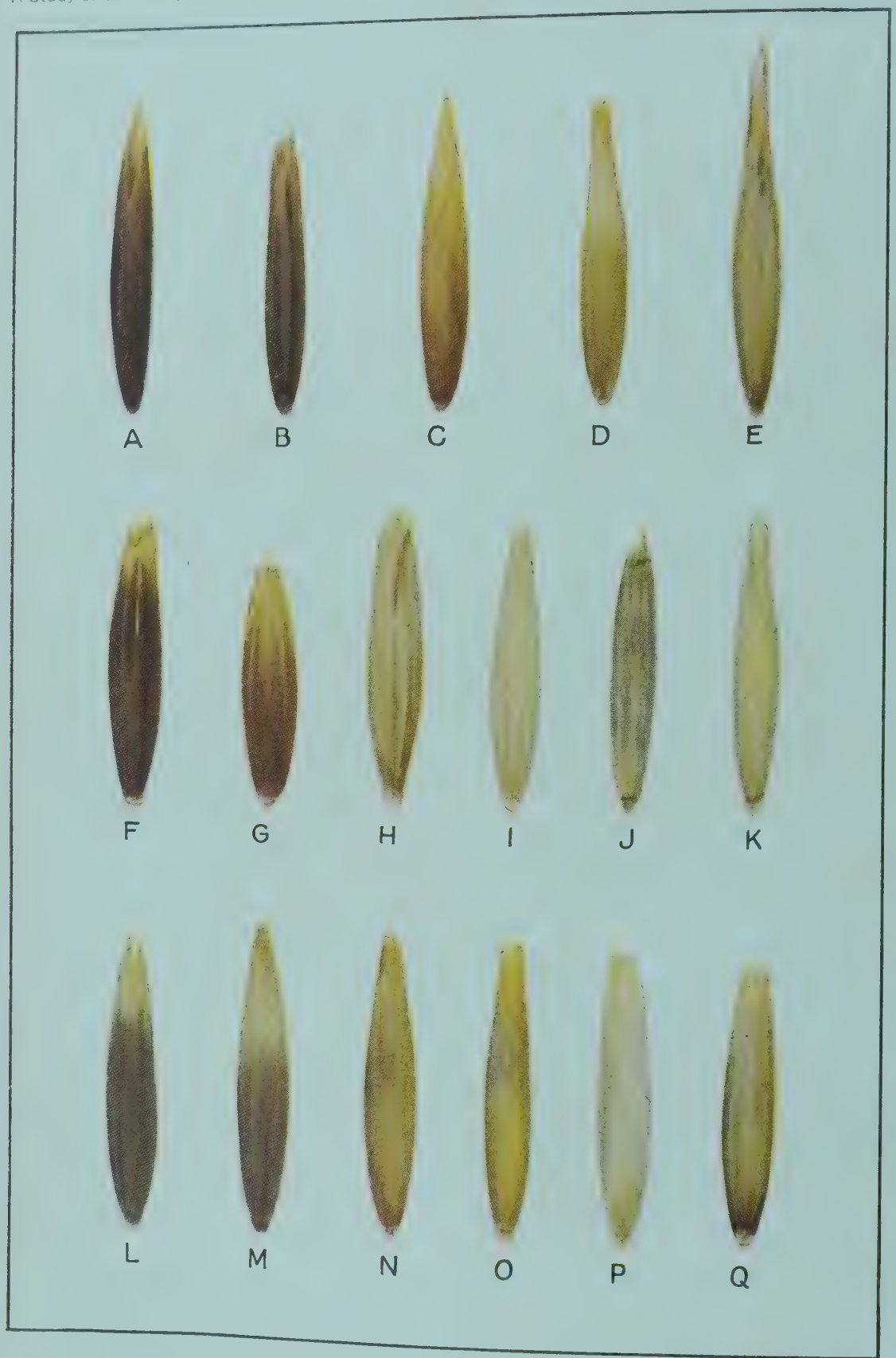


A.—Twisted awns. These awns from Kansas No. 5211 are long, stout, and strongly geniculate with a strong and evident twisting of the basal portion. B.—Nontwisted long awns from Kansas No. 6090. C.—Nontwisted short awns from Kansas No. 5220, varying from a mere point to 15 mm. in length. D.—Awnless condition, from Kansas No. 5211.


A
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Q

ment of agronomy of the Kansas Agricultural Experiment Station give the following histories and descriptions for these strains of Burt oats:

Kansas No. 5020 (Cereal Investigations No. 293) was obtained in 1904 from the cooperative field station of the United States Department of Agriculture and the Kansas Agricultural Experiment Station, at McPherson, Kans. The description of this variety as received at Manhattan was "gray and brown oats of good grade." This strain has been grown in the crop improvement nursery at Manhattan since 1911. In 1919 the general description [21] given the strain was "awnless, smooth and pubescent grains, light yellow to brown in color and with both *sativa* and *sterilis* articulations." The description of the mass color of this strain in 1920 was "pale buckthorn brown."

Kansas No. 5211 (C. I. No. 1917) was received from Prof. T. A. Kiesselbach, of the Nebraska Agricultural Experiment Station, in 1918. This strain was described in 1919 as having "weak awns, smooth and pubescent grains, light red to brown in color and both the *sterilis* and *sativa* types of articulation." The mass color description for this strain was "light buckthorn brown."

Kansas No. 5219 (C. I. No. 2023) was received from Albert Goenner, a farmer of Zenda, Kingman County, Kans., in March, 1920. The selections were made from the original sample of seed as obtained from Mr. Goenner. The mass color description of this variety was "primuline yellow."

Kansas No. 5220 (C. I. No. 293) was received from the Belle Fourche Experiment Farm of the Office of Western Irrigation Agriculture, Bureau of Plant Industry, United States Department of Agriculture, at Newell, S. Dak., in 1916. Although this strain carries the same Cereal Investigations number (293) as Kansas No. 5020, it may have a different origin and history. Burt, C. I. No. 293, is known to vary widely in general appearance at the different stations at which it is being grown. The 1919 description of Kansas No. 5220, as grown at the Kansas station, was "weak awns, smooth and pubescent grains, light yellow to brown in color, with the *sterilis* and *sativa* types of articulation." This strain of Burt oats had a higher proportion of dark-colored florets than any other strain used in these experiments. The description of mass color is "dark buckthorn brown."

Kansas No. 6004 (C. I. No. 1918) originated as a selection from Burt, Kansas No. 5020. The selection was made at the Kansas Agricultural Experiment Station in 1910. The 1919 description for this strain was "awnless, smooth grains, yellow to dark red in color, and *sativa* and *sterilis* articulation." The description of mass color for the strain was "cream color."

Kansas No. 6052 (C. I. No. 1919) also is a selection from Kansas No. 5020, made at the Kansas station in 1911. In 1919 the strain was described as "awnless, smooth grained, light yellow to dark red in color, having both *sterilis* and *sativa* types of articulation." About 98 per cent of the kernels were of a light color. The mass color of this strain was "buff yellow."

Kansas No. 6076 (C. I. No. 1920) originated as a selection from Burt, Kansas No. 5020. This selection was made at the Kansas Agricultural Experiment Station in 1912. The 1919 description for this selection was "awnless, smooth grains, light yellow to brown in color and both the *sativa* and *sterilis* types of articulation." About 75 per cent of the kernels were yellow in color. The description of the mass color for this strain was "maize yellow."

Kansas No. 6090 (C. I. No. 1921) was received in 1918 from Prof. L. C. Burnett, of the Iowa Agricultural Experiment Station. The Iowa nursery row number was 1711, and the seed was described as having "dark kernels." The description of the strain as grown at the Kansas station in 1919 was "awnless, smooth grained, light red to dark brown in color with about 75 per cent of dark kernels, and both the

[21] In this and the following descriptions the word "smooth" means glabrous or without pubescence; the word "grains" means the florets or more particularly the lemmas; and the word "articulation" means disjunction either by disarticulation or by fracture of the rachilla segment. The name "*sterilis*" refers to the cultivated derivatives of that species which in this paper are grouped under the specific name *byzantina*. The color descriptions were made by Mr. S. Fred Prince, biological artist at the Kansas Agricultural Experiment Station. The terms used are those given by Ridgway (*110*) for the corresponding colors.

EXPLANATORY LEGEND FOR PLATE 4

Lemma colors of three strains of Burt oat in 1919. A to E.—Kansas No. 6090: A, black; B, dark brown; C, red; D, reddish yellow; E, red. F to K.—Kansas No. 5219: F, dark brown; G, light brown; H, red; I, yellowish-white; J, gray; K, white. L to Q.—Kansas No. 5220: L, dark brown; M, light brown; N, red; O, yellow; P, white; Q, red.

sativa and *sterilis* types of articulation." The description of mass color for this strain was "mummy brown."

Kansas No. 6094 (C. I. No. 1923) also was received from Prof. L. C. Burnett, of the Iowa Agricultural Experiment Station. Professor Burnett has given the following history and description for this strain: "A pure-line selection made from Burt by Norton before 1906, at the Funk Brothers' Farm, Bloomington, Ill. For many years this was the only variety of Burt at Ames and still is a very good line." The following description was given this strain as grown at the Kansas station in 1919: "Awned, smooth and pubescent grains, light yellow to brown in color and with both *sativa* and *sterilis* types of articulation." The mass color of the florets of this strain was described as "pale buckthorn brown."

EXPERIMENTAL METHODS

The previous observations of the writers had shown marked variability in the Burt oat and arrangements were made for studying the problem cooperatively. Experiments were started at the Akron Field Station and at the Kansas Agricultural Experiment Station in the spring of 1920. In order to coordinate the experiments at the two stations experiments with the Akron selections were discontinued and only the Kansas strains described above were used.

METHODS IN 1920

Kernels were selected individually from each of the nine different lots of bulk seed described under "Experimental material." The attempt was made to select from each of the nine strains about 25 kernels representing each of the recognized phases of the five characters studied. These characters and the number of different phases originally recognized were as follows: Spikelet disarticulation, 3 phases; floret disjunction, 2 phases; basal hairs, 3 phases; awns, 3 phases; lemma color, 6 phases. It was not possible to find so many as 25 primary kernels representing some phases of some characters, while more than 25 were readily obtained in other cases. Only primary or lower kernels to the spikelets were chosen for study. Somewhere between 2,000 and 2,500 kernels were individually selected. About one-third of this material was retained for sowing at the Kansas station and the other two-thirds taken to Akron for sowing there.

At Akron, Colo., those kernels from each strain which represented one phase of each of the five characters were grouped together and grown as a single class. Each plant in each class was numbered at maturity and handled individually thereafter. At the Kansas station each kernel was described individually and given a pedigree number before sowing. With this exception the method of procedure at the two stations differed in minor details only.

There was a slight difference in the manner of describing the original kernels at the two stations. At Akron the nature of the awns was described for each kernel, while at Manhattan only the presence or absence of awns was noted. At Akron only a few of the original lower kernels were classified as having an intermediate type of spikelet disarticulation, while at Manhattan they were separated rather equally into the three groups based on the manner of disarticulation. At Akron a color class, containing variegated kernels, was recognized in 1920, while no such class was recognized at Manhattan.

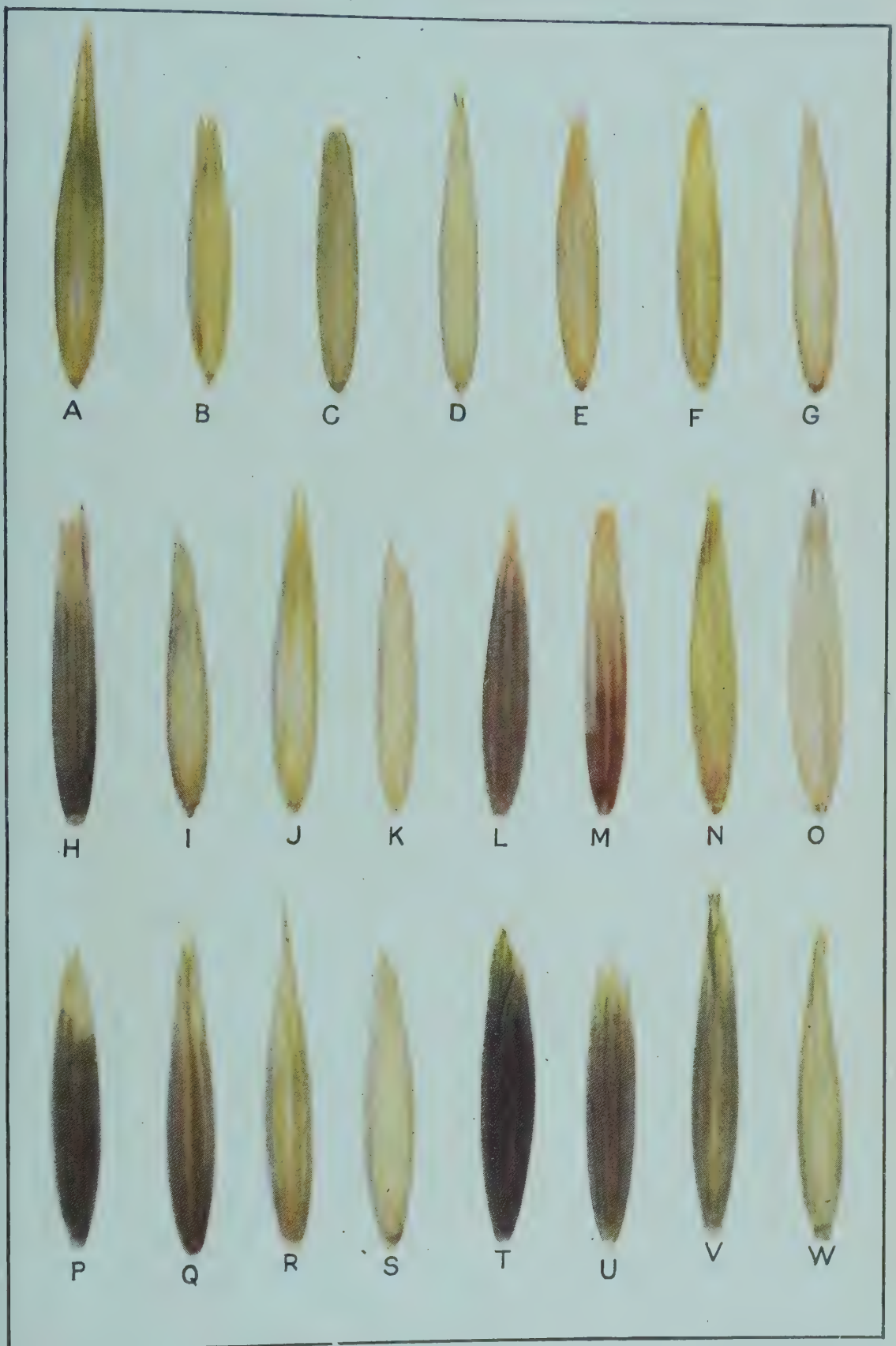
With one exception, seedlings of all strains were made at the Akron Field Station and the Kansas Agricultural Experiment Station in 1920. No seedings of kernels from the Kansas strain No. 5219 were made at Akron. The methods of seeding at the two stations were very similar. Sowing at Akron was done on April 29 and 30 inside a screened shelter, in 10-inch rows with the plants 4 inches apart in the rows. At Manhattan, Kans., sowings were made on April 15, the late spring having made sowing at the normal time impossible. At that station the kernels were spaced 6 inches apart in 12-inch rows.

Before harvest the plants grown at Akron were numbered individually and notes were taken on the number and height of culms, number of panicles, length of main panicle, and date of heading. At Manhattan notes on date of heading and ripening were recorded.

At Akron at harvest time the plants representing each of the score or more

EXPLANATORY LEGEND FOR PLATE 5

Lemma colors of six strains of Burt oat in 1919. A to D.—Kansas No. 6004: A, light red; B, yellow; C, light red; D, white. E to G.—Kansas No. 6052: E, light red; F, yellow; G, white. H to K.—Kansas No. 6076: H, dark brown; I, red; J, yellow; K, white. L to O.—Kansas No. 5020: L, dark brown; M, light brown; N, reddish yellow; O, white. P to S.—Kansas No. 5211: P, black; Q, dark brown; R, red; S, white. T to W.—Kansas No. 6094: T, black; U, dark brown; V, grayish red; W, yellowish white.


A
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W

of character phases in each of the eight strains were pulled and tied together in a bundle. When cured the heads were removed, labeled with the strain number, the class or character phase, and the individual plant number, and shipped to Manhattan for study.

The general plan was to describe at least 25 primary kernels from each plant grown in 1920. Usually all of the primary kernels on the main panicle were described; but where the main panicle did not provide 25 kernels the additional number required was taken from a secondary panicle. The secondary kernels of the spikelets were not described as such in this study but only the method of their disjunction. The kernels were taken from the panicle in a definite order from the base to the top. It was thought that some definite and progressive variation might exist within the panicle, but the results obtained do not indicate such an arrangement. The same characters described in the parental material were used in the descriptions of the progeny kernels of the 1920 crop, namely, (1) spikelet disarticulation; (2) floret disjunction; (3) basal hairs; (4) awns; and (5) lemma color.

Special forms were devised for recording descriptions of the kernels, check marks being made under the proper headings. There were slight differences in the descriptions of the kernels grown at the two stations because the Manhattan material was described first and as the work progressed several minor changes were made.

At first no distinction was made between the abundant long and the abundant midlength subclasses of basal hairs. This distinction was first made toward the end of the study of the 1920 Manhattan material and was continued throughout the description of the Akron material. Shortly after the descriptions of the Akron material was started it appeared advisable to divide the brown color class into dark brown and light brown. No intermediate subclass in floret disjunction was recognized in describing the original kernels, but it was used when describing the kernels of plants grown in 1920.

The percentage of kernels described as being intermediate (heterofracture) for floret disjunction was much higher in the 1920 crop from Akron than in the 1920 crop grown at Manhattan. This may be partly owing to differences in individual judgment, the Manhattan and the Akron material in 1920 being described by two different persons.

METHODS IN 1921

In planning the 1921 experiments it was decided to grow all of the classes and subclasses of kernels representing distinct individual characters and as many different combinations of these as possible. The kernels from each plant grown in 1920 were carefully examined and as complete an array as possible of these characters and combinations of characters was chosen from each of the nine parental strains for sowing in 1921. In making these selections never less than 5 nor more than 10 kernels of a single subclass from any one plant were sown. For example, if in studying floret disjunction in the 25 kernels from plant No. 5211–5–10 (Kansas strain No. 5211, group 5, plant No. 10) 15 kernels were designated by disarticulation and the other 10 kernels by basifracture, probably 10 kernels of each of these two types of floret disjunction were selected for growing. Those of one disjunction type would be grown in 1921 as No. 5211–5–10–1 and those of the other type as 5211–5–10–2. All of the kernels representing a given class or subclass sown in the 1921 experiments at Akron came from the same parent plant. As a rule but few plants derived from any one original strain and bearing the same classification were used for seed, as otherwise the material would soon have become too extensive.

The same general plan was followed in making the kernel selections from the 1920 plants grown at Manhattan, although some classes were chosen for studying the inheritance of a particular character, or combination of characters, without describing all of the other characters of the kernels.

Approximately 800 kernels were selected and sown in the 1921 experiments at Akron. The weather was very dry and grasshoppers did considerable damage to the plants.

Between 500 and 600 kernels were selected, described, and sown in the 1921 experiments at Manhattan. Weather conditions were favorable and the crop made a normal growth. However, a serious infestation of chinch bugs occurred in late June and early July and greatly checked the growth of the plants, with the result that a considerable percentage of the kernels failed to reach their full development.

The general procedure in conducting the 1921 experiments was the same at the two stations. The same notes on plant characters were taken as in 1920.

The plants grown at Akron in 1921 were packed in boxes and mailed to Manhattan, where the material from both stations was described.

EXPERIMENTAL RESULTS

About 60,000 kernels were examined in the course of this study, but space will not permit the inclusion of more than the summary tables of the results obtained. The data will be discussed for each character separately. The five characters are spikelet disarticulation, floret disjunction, basal hairs, awns, and lemma color, as described under experimental methods.

SPIKELET DISARTICULATION

The disarticulation of the spikelet from the lower segment of the rachilla is one of the fundamental characters in classifying oats. In this study disarticulation was described as resulting from "abscission," "semiabscission," or "fracture." Kernels having a clean, sharp separation with a prominent basal cavity with calloused margins, as found in Red Rustproof and other typical varieties of *Avena byzantina*, were described as disarticulating by abscission. This type of separation is illustrated in Plate 1, A. Those kernels having the characteristic roughish fracture and pointed base shown in Plate 1, C without smooth scar tissue or cavity, and generally associated with *Avena sativa* varieties such as Swedish Select were described as disarticulating by fracture. Kernels having a poorly or only partially developed basal cavity with rough tissue surrounding it were described as resulting from semiabscission. A kernel having this basal form is shown in Plate 1, B. The results from the studies of 1920 and 1921 are presented separately.

In selecting the original kernels from the bulk material preparatory to starting these experiments, only the two types, abscission and fracture, were chosen in any considerable number from any of the strains. A few kernels disarticulating by semiabscission, however, were sown at each station. At the Akron station so few kernels of the semiabscission class were selected in any of the strains, with the exception of Kansas No. 5211, that these few kernels were not classified separately but were included with those in which spikelet disarticulation was by abscission.

RESULTS IN 1920

The detailed and summarized results of the study of spikelet disarticulation in 1920 are given in Table I.

The data presented in Table I indicate significant differences among the strains. In only six strains at Manhattan and in seven out of eight at Akron were parental kernels found which disarticulated by abscission. This clearly indicates that variation exists among different Burt oat strains with respect to this character.

Kernels in which spikelet disarticulation resulted from abscission tend to produce progeny of the same type. Parental kernels described as disarticulating by fracture produced progeny of which about three-fourths disarticulated by fracture. Kernels in which spikelet disarticulation was classed as resulting from semiabscission were found in appreciable percentages in the progenies of all three parental classes.

Kernels having the prominent basal cavity associated with abscission were found in the progeny of fracturing parental kernels, which have no cavity, in six strains at each station. Two strains, Kansas Nos. 5219 and 5220, at Manhattan, and one strain, Kansas No. 6004, at Akron, produced no kernels in which spikelet disarticulation was described as by fracture in the progenies of parental kernels disarticulating by abscission and semiabscission combined.

The disarticulation resulting from fracture was very definitely inherited. At both stations, in all strains, with the exception of Kansas No. 6090 grown at Akron, the parental kernels in which the spikelet disarticulation was described as fracture produced progeny of which 60 per cent or more was described as disarticulating by fracture. In general the results obtained show a rather strong correlation between parent and progeny in spikelet disarticulation.

From the data in Table I it is evident that spikelet disarticulation is inherited in a definite manner. The summarized data indicate that there was a strong tendency for the kernels disarticulating by abscission and by fracture to transmit these characters to their progeny. The kernels in the class disarticulating by semiabscission tended to produce progeny almost equally divided among the three spikelet disarticulation types.

RESULTS IN 1921

Table II presents the data obtained at Akron in 1921 on the inheritance of spikelet disarticulation. (Since the plants of Burt oats at Manhattan did not attain full development because of the ravages of chinch bugs, accurate classification with regard to spikelet

TABLE I.—*Data on inheritance of three different methods of spikelet disarticulation in the progeny of selected kernels of each of nine strains of the Burt oat when grown at the Akron Field Station and at the Kansas Agricultural Experiment Station in 1920*

Strain number and method of disarticulation in parent kernels	Kernels in progeny					
	Number disarticulating by—			Percentage disarticulating by—		
	Abscission	Semiabscission	Fracture	Abscission	Semiabscission	Fracture
AKRON FIELD STATION						
Kansas No. 5020:						
Abscission	419	506	71	42.1	50.8	7.1
Semiabscission						
Fracture	26	734	1,205	1.3	37.4	61.3
Kansas No. 5211:						
Abscission	3,439	543	276	80.8	12.7	6.5
Semiabscission	291	75	2	79.1	20.4	.5
Fracture	637	1,176	3,298	12.5	23.0	64.5
Kansas No. 5220:						
Abscission	91	109	20	41.4	49.5	9.1
Semiabscission						
Fracture	10	404	1,000	.7	28.6	70.7
Kansas No. 6004:						
Abscission	77	96	0	44.5	55.5	0
Semiabscission						
Fracture	24	54	913	2.4	5.5	92.1
Kansas No. 6052:						
Fracture	0	118	3,242	0	3.5	96.5
Kansas No. 6076:						
Abscission	60	194	113	16.3	52.9	30.8
Semiabscission						
Fracture	0	213	1,471	0	12.6	87.4
Kansas No. 6090:						
Abscission	702	650	118	47.8	44.2	8.0
Semiabscission						
Fracture	418	667	1,013	19.9	31.8	48.3
Kansas No. 6094:						
Abscission	621	133	76	74.8	16.0	9.2
Semiabscission						
Fracture	109	301	1,333	6.2	17.3	76.5
All 8 strains:						
Abscission	5,409	2,231	674	65.1	26.8	8.1
Semiabscission	291	75	2	79.1	20.4	.5
Fracture	1,224	3,667	13,475	6.6	20.0	73.4
KANSAS AGRICULTURAL EXPERIMENT STATION						
Kansas No. 5020:						
Abscission	145	144	45	43.4	43.1	13.5
Semiabscission	29	74	257	8.0	20.6	71.4
Fracture	35	178	736	3.7	18.8	77.5
Kansas No. 5211:						
Abscission	528	148	65	71.2	20.0	8.8
Semiabscission	148	142	139	34.5	33.1	32.4
Fracture	123	142	624	13.8	16.0	70.2
Kansas No. 5219:						
Abscission	456	275	0	62.4	37.6	0
Semiabscission	237	340	61	37.1	53.3	9.6
Fracture	44	153	338	8.2	28.6	63.2
Kansas No. 5220:						
Abscission	59	7	0	89.4	10.6	0
Semiabscission	16	39	130	8.6	21.1	70.3
Fracture	26	123	479	4.1	19.6	76.3
Kansas No. 6004:						
Semiabscission	0	17	25	0	40.5	59.5
Fracture	0	1	894	0	.1	99.9
Kansas No. 6052:						
Semiabscission	0	17	2	0	89.5	10.5
Fracture	0	4	1,398	0	.3	99.7
Kansas No. 6076:						
Semiabscission	0	2	6	0	25.0	75.0
Fracture	0	88	899	0	8.9	91.1
Kansas No. 6090:						
Abscission	232	182	17	53.8	42.2	4.0
Semiabscission	57	145	111	18.2	46.3	35.5
Fracture	58	80	479	9.4	13.0	77.6

TABLE I.—*Data on inheritance of three different methods of spikelet disarticulation in the progeny of selected kernels of each of nine strains of the Burt oat when grown at the Akron Field Station and at the Kansas Agricultural Experiment Station in 1920*—Continued

Strain number and method of disarticulation in parent kernels	Kernels in progeny					
	Number disarticulating by—			Percentage disarticulating by—		
	Abscission	Semiabscission	Fracture	Abscission	Semiabscission	Fracture
KANSAS AGRICULTURAL EXPERIMENT STATION—continued						
Kansas No. 6094:						
Abscission	150	3	13	90.4	1.8	7.8
Semiabscission	25	63	73	15.5	39.1	45.4
Fracture	3	205	348	.5	36.9	62.6
All 9 strains:						
Abscission	1,570	759	140	63.6	30.7	5.7
Semiabscission	512	839	804	23.8	38.9	37.3
Fracture	289	974	6,195	3.9	13.0	83.1
BOTH STATIONS						
Abscission	6,979	2,990	814	64.7	27.7	7.6
Semiabscission	803	914	806	31.8	36.2	32.0
Fracture	1,513	4,641	19,670	5.8	18.0	76.2

disarticulation was impossible and no data on this character are included.) The abscission type bred true in 92.4 per cent of the kernels grown from parental kernels described as disarticulating by this method.

The parental kernels in which spikelet disarticulation was classed as resulting from semiabscission, produced progeny containing approximately three individuals in which spikelet disarticulation occurred by semiabscission or fracture to one by abscission. The parental kernels in which spikelet disarticulation was described as fracture, produced progeny of which the spikelet disarticulation was 64.3 per cent by fracture, 33.2 per cent by semiabscission, and 2.5 per cent by abscission.

It would appear from the 1921 data on the inheritance of spikelet disarticulation that in the Burt oat the spikelet disarticulation by fracture is dominant and that by abscission recessive. The class here described as resulting by semiabscission possibly represents the heterozygous group. These results seem to be in general agreement with those of Surface (*127*), Love and Craig (*70*), and Fraser (*37*), who studied the inheritance of the basal scar as an expression of this character.

The results obtained agree with those of previous investigators and indicate that spikelet disarticulation behaves as a simple monohybrid. Fracture or the absence of basal cavity probably is dominant, or at least partially so, over abscission or presence of a basal cavity which seems to behave as a recessive.

Etheridge (*33*) believed that the basal cavity is of little use in classifying oat varieties. The results of many investigators indicate that this character is of distinct value in classifying oats and that it is more or less constant in its breeding behavior. This character has been much used in distinguishing oat varieties of the *Avena byzantina* group from those of the *sativa* group and it is thought to be of more value than the statement of Etheridge would indicate.

FLORET DISJUNCTION

The floret disjunction classes previously described are disarticulation (as in *A. sativa*), heterofracture, and basifracture (as in *A. byzantina*). These three ways by which floret disjunction takes place are illustrated in Plate 1, D to F, inclusive. The term heterofracture was used for the method of disjunction of those florets or kernels in which the rachilla segment broke variously at or near the midpoint, as shown in Plate 1, E. In these cases part of the rachilla remained with the primary kernel of the spikelet and part of it broke off with the secondary kernel.

TABLE II.—*Data on inheritance of three different methods of spikelet disarticulation in the progeny of selected kernels of each of eight strains of the Burt oat when grown at the Akron Field Station in 1921*

Strain number and method of disarticulation in parent kernels	Kernels in progeny					
	Number disarticulating by—			Percentage disarticulating by—		
	Abscission	Semiabscission	Fracture	Abscission	Semiabscission	Fracture
Kansas No. 5020:						
Semiabscission	266	449	34	35.5	60.0	4.5
Fracture	0	108	195	0	35.6	64.4
Kansas No. 5211:						
Abscission	1,135	111	2	90.9	8.9	.2
Semiabscission	41	418	108	7.2	73.7	19.1
Fracture	73	337	926	5.5	25.2	69.3
Kansas No 5220:						
Abscission	91	0	0	100.0	0	0
Semiabscission	1	144	6	.6	95.4	4.0
Fracture	0	144	180	0	44.4	55.6
Kansas No. 6004:						
Fracture	0	0	29	0	0	100.0
Kansas No. 6052:						
Semiabscission	0	73	4	0	94.8	5.2
Fracture	0	114	147	0	43.7	56.3
Kansas No. 6076:						
Abscission	0	0	6	0	0	100.0
Semiabscission	53	158	44	20.8	61.9	17.3
Fracture	0	14	262	0	5.1	94.9
Kansas No. 6090:						
Abscission	345	20	0	94.5	5.5	0
Semiabscission	63	117	8	33.5	62.2	4.3
Fracture	0	112	71	0	61.2	38.8
Kansas No 6094:						
Abscission	130	1	0	99.2	0.8	0
Semiabscission	109	21	9	78.4	15.1	6.5
Fracture	0	151	87	0	63.4	36.6
All strains:						
Abscission	1,701	132	8	92.4	7.2	.4
Semiabscission	533	1,380	213	25.1	64.9	10.0
Fracture	73	980	1,897	2.5	33.2	64.3

STUDY IN 1920

Table III presents the data on floret disjunction for each strain grown at Akron and Manhattan in 1920. The numbers of kernels studied and percentages falling into each disjunction class are given. The combined data for all strains at each station and at both stations are shown also. The parental kernels sown in 1920 were separated into only two classes for disjunction. The kernels properly representing the intermediate class (heterofracture) probably went mostly into the basifracture class.

A study of the data on floret disjunction in the 1920 crop indicates that the *sativa* type (which disarticulates) is much more stable in breeding behavior than the *sterilis* type (which separates by basifracture). More than 67 per cent of the progeny kernels produced at both Akron and Manhattan from parental kernels disjoining by disarticulation were described as disarticulating, slightly less than 23 per cent as separating by heterofracture, and only 10 per cent as separating by basifracture.

Only 18.3 per cent of progeny kernels in which disjunction took place by basifracture were produced from parental kernels so classed. More than twice as many of the 1920 progeny from kernels of this class, or 47.1 per cent, were described as disjoining by disarticulation as by basifracture, while in 34.6 per cent disjunction was described as by heterofracture.

Comparatively few of the kernels in which floret disjunction was classed as taking place by basifracture bred true in 1920. These data indicate that in this material the disarticulation form is more stable in breeding behavior than the basifracture form of disjunction.

The summarized data for each of the two stations show that the percentage of progeny kernels disjoining by disarticulation, and produced from parental kernels so classed, was about 66 per cent at Akron and 73 per cent at Manhattan. The percentage of progeny kernels disjoining by heterofracture and grown from parent kernels in which disjunction was classed as by disarticulation and as by basifracture varied considerably at the

two stations. This probably was partly owing to the fact that the descriptions of the kernels grown at the two stations in 1920 were made by different persons. The percentage of basifracture kernels in the progeny of parental kernels described as disjoining by disarticulation was much lower at Akron, 8 per cent, than at Manhattan, 21.7 per cent.

In the progeny of parental kernels in which disjunction was described as by basifracture, 13.5 per cent of the kernels grown at Akron and 31.8 per cent of the kernels grown at Manhattan were so described. In the progenies of parental kernels in which disjunction was described as by basifracture there were about 61 per cent by disarticulation in the Manhattan material and about 42 per cent in the Akron material.

The highest percentage of progeny kernels of which floret disjunction was described as by basifracture, produced from parental kernels so classed, was found in strain Kansas No. 5211 at Akron and in strain No. 5219 at Manhattan. Kansas No. 5219 was not grown at Akron. The percentage of disjunction by disarticultion in the progeny of parental kernels in which disjunction was described as by basifracture was not far below 60 per cent in any of the strains at either station, with the exception of Kansas No. 6090, grown at Akron, and Kansas No. 5219, grown at Manhattan. In Kansas No. 6052, grown at Akron, and Kansas Nos. 6052 and 6076, grown at Manhattan, less than 5 per cent of the progeny kernels of parental kernels in which floret disjunction was described as by basifracture were so classed. In each of these three cases, in about 90 per cent or more of the progeny of the basifracture parental type, disjunction was described as by disarticulation. The comparatively small number of individuals of each of these strains, however, makes it unwise to consider these results conclusive.

An examination of the data for each of the strains at both stations indicates that of the disjunction types the lowest percentages of progeny described as disarticulating, from parental kernels so described, were produced in those strains which had the highest percentages of progeny disjunction by basifracture produced from parents of that type. In most of the strains grown at each station approximately two-thirds, and in many cases three-fourths or more, of the progeny from parental kernels disjoining by disarticulation were described as disarticulating.

The results of the 1920 studies on floret disjunction clearly indicate that the form resulting from basifracture in all of the strains under observation was much less stable in breeding behavior than was the form resulting from disarticulation. A comparatively small percentage of the parental kernels in which disjunction took place by basifracture produced progeny so described, while in most strains at both stations two-thirds or more of the progeny kernels from parental kernels in which disjunction was described as by disarticulation were of this type.

STUDY IN 1921

Floret disjunction in the progeny kernels grown in 1921 at Akron was described in the same way as in 1920. (Chinch bugs damaged the oat plants at Manhattan to such an extent that it is impossible to include data for the 1921 crop at that place.) The results obtained in 1921, presented in Table IV, are in general agreement with those obtained in 1920.

An examination of the data shows that a comparatively small percentage of the kernels in which disjunction was classed as by basifracture bred true. In each of the five strains where progenies were produced from kernels so described less than 20 per cent of the progeny kernels in any strain disjoined by basifracture. This indicates that in the Burt oat used in these experiments disjunction by basifracture is very unstable in its breeding behavior, producing larger percentages of progeny in which disjunction takes place by disarticulation and by heterofracture than by basifracture even in the second year of pedigree culture.

The kernels in which disjunction was described as by heterofracture in 1920 produced progenies in 1921 of which approximately 45 per cent of the kernels disjoined by heterofracture, 44 per cent by disarticulation, and 11 per cent by basifracture. It is possible that in describing the 1920 material a larger number of kernels of which disjunction was genetically by disarticulation than of those in which it was genetically by basifracture was classed as by heterofracture.

The results obtained in the progenies from kernels of which disjunction was classed as by disarticulation in 1920 indicate that disarticulation is comparatively stable in breeding behavior. The progeny kernels produced in 1921 from the kernels of which disjunction was by disarticulation showed about 74 per cent disjoining by disarticulation, 22 per cent by heterofracture, and less than 5 per cent by basifracture.

TABLE III.—*Data on inheritance of three different methods of floret disjunction in the progeny of selected kernels of each of nine strains of the Burt oat when grown at the Akron Field Station and at the Kansas Agricultural Experiment Station in 1920*

Strain number and method of disjunction in parent kernels[a]	Kernels in progeny					
	Number disjoining by—			Percentage disjoining by—		
	Basi-fracture	Hetero-fracture	Disartic-ulation	Basi-fracture	Hetero-fracture	Disartic-ulation
AKRON FIELD STATION						
Kansas No. 5020:						
Basifracture	32	207	293	6.0	38.9	55.1
Disarticulation	76	568	1,785	3.1	23.4	73.5
Kansas No. 5211:						
Basifracture	503	1,535	814	17.6	53.8	28.6
Disarticulation	804	2,447	3,156	12.5	38.2	49.3
Kansas No. 5220:						
Disarticulation	175	413	1,046	10.7	25.3	64.0
Kansas No. 6004:						
Basifracture	9	17	52	11.5	21.8	66.7
Disarticulation	19	119	948	1.7	11.0	87.3
Kansas No. 6052:						
Basifracture	8	27	302	2.4	8.0	89.6
Disarticulation	121	273	2,629	4.0	9.0	87.0
Kansas No. 6076:						
Basifracture	88	93	404	15.0	15.9	69.1
Disarticulation	104	172	1,190	7.1	11.7	81.2
Kansas No. 6090:						
Basifracture	143	684	358	12.1	57.7	30.2
Disarticulation	212	742	1,429	8.9	31.1	60.0
Kansas No. 6094:						
Basifracture	76	257	477	9.4	31.7	58.9
Disarticulation	107	498	1,158	6.1	28.2	65.7
All strains:						
Basifracture	859	2,818	2,700	13.5	44.2	42.3
Disarticulation	1,618	5,232	13,341	8.0	25.9	66.1
KANSAS AGRICULTURAL EXPERIMENT STATION						
Kansas No. 5020:						
Basifracture	65	12	172	26.1	4.8	69.1
Disarticulation	24	6	111	17.0	4.3	78.7
Kansas No. 5211:						
Basifracture	152	29	269	33.8	6.4	59.8
Disarticulation	254	78	574	28.0	8.6	63.4
Kansas No. 5219:						
Basifracture	381	101	425	42.0	11.1	46.9
Disarticulation	244	49	452	32.7	6.6	60.7
Kansas No. 6004:						
Basifracture	13	8	91	11.6	7.1	81.3
Disarticulation	1	1	89	1.1	1.1	97.8
Kansas No. 6052:						
Basifracture	6	4	125	4.4	3.0	92.6
Disarticulation	14	7	328	4.0	2.0	94.0
Kansas No. 6076:						
Basifracture	1	1	80	1.2	1.2	97.6
Disarticulation	13	1	127	9.2	0.7	90.1
Kansas No. 6090:						
Basifracture	79	12	130	35.8	5.4	58.8
Disarticulation	109	31	447	18.6	5.3	76.1
Kansas No. 6094:						
Basifracture	26	6	87	21.9	5.0	73.1
Disarticulation	102	9	434	18.7	1.7	79.6
All strains:						
Basifracture	723	173	1,379	31.8	7.6	60.6
Disarticulation	761	182	2,562	21.7	5.2	73.1
BOTH STATIONS						
Basifracture	1,582	2,991	4,079	18.3	34.6	47.1
Disarticulation	2,379	5,414	15,903	10.0	22.9	67.1

[a] None of the parental kernels sown in 1920 was described as having intermediate or heterofracture disjunction.

TABLE IV.—*Data on inheritance of three different methods of floret disjunction in the progeny of selected kernels of each of eight strains of the Burt oat when grown at the Akron Field Station in 1921*

Strain number and method of disjunction in parent kernels	Kernels in progeny					
	Number disjoining by—			Percentage disjoining by—		
	Basifracture	Heterofracture	Disarticulation	Basifracture	Heterofracture	Disarticulation
Kansas No. 5020:						
Basifracture	3	7	21	9.7	22.6	67.7
Heterofracture	10	96	84	5.3	50.5	44.2
Disarticulation	33	169	629	4.0	20.3	75.7
Kansas No. 5211:						
Basifracture	84	162	181	19.7	37.9	42.4
Heterofracture	151	408	442	15.1	40.7	44.2
Disarticulation	92	469	1,162	5.3	27.2	67.5
Kansas No. 5220:						
Basifracture	2	9	34	4.4	20.0	75.6
Heterofracture	6	28	70	5.8	26.9	67.3
Disarticulation	15	40	362	3.6	9.6	86.8
Kansas No. 6004:						
Disarticulation	1	2	26	3.4	6.9	89.7
Kansas No. 6052:						
Heterofracture	0	0	7	0	0	100.0
Disarticulation	6	28	279	1.8	8.5	89.7
Kansas No. 6076:						
Basifracture	5	28	16	10.2	57.1	32.7
Heterofracture	0	20	5	0	80.0	20.0
Disarticulation	30	124	309	6.5	26.8	66.7
Kansas No. 6090:						
Heterofracture	43	300	221	7.6	53.2	39.2
Disarticulation	4	26	142	2.3	15.1	82.6
Kansas No. 6094:						
Basifracture	2	34	49	2.4	40.0	57.6
Heterofracture	8	64	57	6.2	49.6	44.2
Disarticulation	9	79	206	3.1	26.9	70.0
All strains:						
Basifracture	96	240	301	15.1	37.7	47.2
Heterofracture	218	916	886	10.8	45.3	43.9
Disarticulation	190	937	3,133	4.5	22.0	73.5

The results of the 1921 experiments on the inheritance of floret disjunction indicate the correctness of the conclusions drawn in 1920 and confirm the statement that floret disjunction by basifracture as observed in these Burt oat strains is very much less stable in breeding behavior than is that by disarticulation.

A review of the data on floret disjunction obtained in these experiments indicates that the disjunction resulting from disarticulation is the more stable in breeding behavior. The results for the two years were similar in this respect. The results obtained by Fraser (*37*), in his cross of Burt (*sterilis* type) × Sixty-Day (*sativa* type) indicated that the *sativa* type of floret disjunction by disarticulation is dominant over the *sterilis* type which results from basifracture and that a single factor difference governs the inheritance of floret disjunction.

The data presented on the inheritance of the floret disjunction in the Burt oat indicates that, to explain the breeding behavior of this character in this variety on a factorial basis, it will be necessary to conduct carefully controlled hybridization experiments using parental material of which the breeding behavior for this character is definitely known.

BASAL HAIRS

In classifying the material from the nine strains of Burt oat used, three quantity classes for basal hairs were recognized, namely, abundant, few, and absent. In describing the progeny kernels of the 1920 crop a subdivision of the first class seemed necessary to allow separation of long and mid-length hairs. As the kernels grown in 1919 and sown in the 1920 experiments were machine threshed, many of the basal hairs were rubbed off and for that reason classification of the original material was subject to error. In some cases kernels which had borne hairs probably lost all or many of them in threshing and an erroneous description resulted.

Kernels described in 1920 as subclass abundant long had a dense growth of long basal hairs which sometimes almost surrounded the base of the lemma (Pl. 2, A). The basal hairs described as abundant midlength (Pl. 2, B) were not so long as those in the abundant long subclass and often they were not produced so abundantly. Kernels described as having few basal hairs usually had only a few short hairs around the base of the kernel (Pl. 2, C), although some kernels bearing a very few long hairs were included. Kernels which bore long hairs usually had them abundantly developed. Kernels bearing no visible hairs were classed as absent (Pl. 1, B, C, E).

STUDY IN 1920

The data on basal hairs from both stations in 1920, presented in Table V, clearly indicate that all of the three parental types segregated to a considerable degree. The abundant class was a little more constant in breeding behavior than either of the others. The class termed few was the least constant. In general, however, each parental class produced progenies having a slightly larger number of kernels of its own class than of either of the other classes.

An examination of the data for each strain shows that in only five of the eight strains grown at Akron were any of the parental kernels described as having abundant hairs. If the percentages of kernels classed as abundant midlength and abundant long produced from the parental kernels in the abundant class are added together for all strains grown at Akron, nearly 85 per cent of the progeny kernels are in the two abundant subclasses. The percentages range from 75 per cent in Kansas No. 6076 to 93.5 per cent in Kansas No. 6090. In all strains grown at Akron in 1920 the parental kernels described as having abundant basal hairs produced progenies in which practically no kernels were classed as having basal hairs absent, although 9.2 per cent of such kernels occurred in Kansas No. 5211. In Kansas No. 5020 only two kernels and in Kansas No. 6076 only one kernel having no basal hairs occurred in progenies of parental kernels having abundant hairs. As a rule the parental kernels classed as having abundant basal hairs were more nearly constant in breeding behavior than either of the other two classes. These data show clearly how few kernels genetically having long hairs were classed as having few hairs.

In many of the strains the kernels classed as having few basal hairs produced a majority of kernels of the abundant hair class. Of all of the strains at both stations parental kernels with few hairs produced only 2.5 per cent of progeny kernels with hairs classed as abundant long and only 5.1 per cent classed as absent, while 44.9 per cent were classed as abundant midlength, and 47.5 per cent as few. The parental kernels classed as having no basal hairs produced 6.8 per cent with hairs described as abundant midlength, and only 0.3 per cent with abundant long hairs, but 36.4 per cent with few, and 56.5 per cent with hairs absent. Of all of the eight strains only 36.4 per cent of the progeny of parental kernels having absent basal hairs were classed as having few hairs.

The summary shows that all parental classes produced some kernels in all of the classes of basal hairs. Those parental kernels classed as having abundant hairs bred true in 76 per cent of the cases, if the abundant long and midlength subclasses are considered together. Parental kernels classed as having few hairs produced progenies consisting very largely of the abundant midlength and few classes. The parental kernels classed as having hairs absent produced progeny of which only 56.5 per cent were so described, while 43.5 per cent bore basal hairs.

STUDY IN 1921

The 1921 data on inheritance of basal hairs are presented in Table VI. No kernels described as having abundant long basal hairs were sown at Manhattan. At Akron these kernels produced progeny of which 95 per cent were like the parents and 5 per cent had abundant midlength hairs. This would indicate that the abundant long class of basal hairs breeds as a recessive. Kernels described as having abundant midlength basal hairs produced progenies of which 53.3 per cent were of that class, about 30 per cent were abundant long, 16 per cent few, and less than 1 per cent absent.

Parental kernels described as having few basal hairs produced progeny of which the basal hairs on less than 1 per cent were classed as abundant long, on about 46 per cent as abundant midlength, on 46 per cent as few, and on about 7 per cent as absent.

The progeny of parental kernels described as having basal hairs absent were distributed about as follows: 62 per cent absent, 36 per cent few, 2 per cent abundant midlength, and less than 1 per cent abundant long.

TABLE V.—*Data on inheritance of basal hairs in 9 strains of Burt oat grown at the Akron Field Station and at the Kansas Agricultural Experiment Station in 1920*

Strain number and basal hairs in parent kernels [a]	Basal hairs in progeny							
	Number of kernels with hairs—				Percentage of kernels with hairs—			
	Abundant		Few	Absent	Abundant		Few	Absent
	Long	Mid-length			Long	Mid-length		
AKRON FIELD STATION								
Kansas No. 5020:								
Abundant	46	52	25	2	36.8	41.6	20.0	1.6
Few	0	127	102	21	0	50.8	40.8	8.4
Absent	5	245	1,006	1,330	.2	9.5	38.9	51.4
Kansas No. 5211:								
Abundant	500	188	47	74	61.8	23.2	5.8	9.2
Few	77	542	905	18	5.0	35.1	58.7	1.2
Absent	21	916	2,643	3,806	.3	12.4	35.8	51.5
Kansas No. 5220:								
Few	0	77	4	0	0	95.1	4.9	0
Absent	0	19	801	733	0	1.2	51.6	47.2
Kansas No. 6004:								
Few	0	115	33	0	0	77.7	22.3	0
Absent	0	27	253	736	0	2.7	24.9	72.4
Kansas No. 6052:								
Absent	0	0	951	2,409	0	0	28.3	71.7
Kansas No. 6076:								
Abundant	0	57	18	1	0	75.0	23.7	1.3
Few	0	0	63	25	0	0	71.6	28.4
Absent	0	5	444	1,438	0	.3	23.5	76.2
Kansas No. 6090:								
Abundant	0	29	2	0	0	93.5	6.5	0
Few	0	467	109	28	0	77.3	18.1	4.6
Absent	0	471	1,128	1,334	0	16.1	38.4	45.5
Kansas No. 6094:								
Abundant	0	264	37	0	0	87.7	12.3	0
Few	0	282	166	0	0	62.9	37.1	0
Absent	0	154	423	1,248	0	8.4	23.2	68.4
All strains:								
Abundant	546	590	129	77	40.7	44.0	9.6	5.7
Few	77	1,610	1,381	92	2.4	50.9	43.7	2.9
Absent	26	1,837	7,649	13,034	.1	8.2	33.9	57.8
KANSAS AGRICULTURAL EXPERIMENT STATION								
Kansas No. 5020:								
Abundant	0	70	56	38	0	42.7	34.1	23.2
Few	0	25	88	13	0	19.9	69.8	10.3
Absent	0	34	569	750	0	2.5	42.1	55.4
Kansas No. 5211:								
Abundant	3	21	55	0	3.8	26.6	69.6	0
Few	2	129	245	31	.5	31.7	60.2	7.6
Absent	55	122	696	700	3.5	7.8	44.2	44.5
Kansas No. 5219:								
Abundant	0	0	21	0	0	0	100.0	0
Few	29	41	25	18	25.7	36.3	22.1	15.9
Absent	0	159	1,166	445	0	9.0	65.9	25.1
Kansas No. 5220:								
Few	0	20	46	9	0	26.7	61.3	12.0
Absent	0	12	280	512	0	1.5	34.8	63.7
Kansas No. 6004:								
Few	0	0	5	20	0	0	20.0	80.0
Absent	0	0	360	552	0	0	39.5	60.5
Kansas No. 6052:								
Absent	0	0	302	1,119	0	0	21.3	78.7
Kansas No. 6076:								
Absent	0	0	409	586	0	0	41.1	58.9
Kansas No. 6090:								
Abundant	0	4	25	1	0	13.3	83.3	3.4
Few	0	84	198	29	0	27.0	63.7	9.3
Absent	0	43	342	635	0	4.2	33.5	62.3

[a] No differentiation was made between the long and midlength subclasses of basal hairs in describing the kernels sown in 1920.

TABLE V.—*Data on inheritance of basal hairs in 9 strains of Burt oat grown at the Akron Field Station and at the Kansas Agricultural Experiment Station in 1920*—Continued

Strain number and basal hairs in parent kernels[a]	Basal hairs in progeny							
	Number of kernels with hairs—				Percentage of kernels with hairs—			
	Abundant		Few	Absent	Abundant		Few	Absent
	Long	Mid-length			Long	Mid-length		
KANSAS AGRICULTURAL EXPERIMENT STATION—continued								
Kansas No. 6094:								
Abundant	0	41	1	0	0	97.6	2.4	0
Few	0	18	48	8	0	24.3	64.9	10.8
Absent	0	63	297	407	0	8.2	38.7	53.1
All strains:								
Abundant	3	136	158	39	.9	40.5	47.0	11.6
Few	31	317	655	128	2.8	28.0	57.9	11.3
Absent	55	433	4,421	5,706	.5	4.1	41.6	53.8
BOTH STATIONS								
Abundant	549	726	287	116	32.7	43.3	17.1	6.9
Few	108	1,927	2,036	220	2.5	44.9	47.5	5.1
Absent	81	2,270	12,070	18,740	.3	6.8	36.4	56.5

[a] No differentiation was made between the long and midlength subclasses of basal hairs in describing the kernels sown in 1920.

The results obtained in the study of the basal-hair character in Burt oat indicate that the abundant long hairs probably are a recessive character. Apparently several factors are involved in the production of the various classes of basal hairs, or, if determined by a single main factor, several modifiers possibly are involved. The data indicate that the absence of basal hairs is partially dominant over their presence in the Burt oat. This conclusion is in accord with the results obtained by Nilsson-Ehle (*91*), Zade (*153*), Surface (*127*), Love and Craig (*70*), and Fraser (*37*). The last named investigator obtained results which are very interesting in this connection. He observed that short basal hairs or no basal hairs are dominant over midlength basal hairs and obtained a ratio in F_2 of three of the former to one of the latter.

AWNS

The awns found in these strains of the Burt oat were classified as twisted, nontwisted, and absent. The nontwisted class was further subdivided into long and short. Data obtained in 1920 and in 1921 are presented separately.

STUDY IN 1920

The material from which the kernels used in starting these experiments was selected was machine threshed and many awns were broken off. For this reason, only the presence or absence of awns was recorded for the kernels sown at Manhattan in 1920. Hence there are no data on the inheritance of awns at the Kansas station in that year. However, the kernels sown at Akron in 1920 were selected with special reference to the awn and were described for this character.

In the classification of the parental material sown at Akron in 1920 only three awn classes were recognized. These were twisted, nontwisted, and absent. In the nontwisted class were included the two subclasses later described in the progeny as long and short. These two subclasses grade into one another in such a way as to make exact classification very difficult. Fraser (*37*) has included them in his "weak" awn class and uses the term "strong" awn to describe the class here called twisted.

Four awn groups were recognized in the classification of the progeny kernels grown at Akron in 1920, namely, twisted, nontwisted long, nontwisted short, and absent (Pl. 3). The twisted awns were similar to, although not as strongly developed as, the type of awn found in the wild species, *Avena fatua* and *A. sterilis*. They are rather stiff, twisted at the base, and more or less geniculate. The twisted lower portion of this awn is composed of alternate stripes of dark and light colored tissue.

TABLE VI.—*Data on inheritance of basal hairs in 9 strains of Burt oat grown at the Akron Field Station and at the Kansas Agricultural Experiment Station in 1921*

Strain number and basal hairs in parent kernels	Basal hairs in progeny							
	Number of kernels with hairs—				Percentage of kernels with hairs—			
	Abundant		Few	Absent	Abundant		Few	Absent
	Long	Mid-length			Long	Mid-length		
AKRON FIELD STATION								
Kansas No. 5020:								
Abundant long	153	10	0	0	93.9	6.1	0	0
Few	0	84	437	47	0	14.8	76.9	8.3
Absent	0	0	175	146	0	0	54.5	45.5
Kansas No. 5211:								
Abundant long	339	16	0	0	95.5	4.5	0	0
Abundant midlength	101	322	79	5	19.9	63.5	15.6	1.0
Few	14	572	203	28	1.7	70.0	24.8	3.4
Absent	8	83	303	1,078	.6	5.6	20.6	73.2
Kansas No. 5220:								
Abundant midlength	0	83	8	0	0	91.2	8.8	0
Few	0	2	232	18	0	.8	92.1	7.1
Absent	0	0	103	120	0	0	46.2	53.8
Kansas No. 6004:								
Absent	0	0	7	22	0	0	24.1	75.9
Kansas No. 6052:								
Few	0	0	62	15	0	0	80.5	19.5
Absent	0	0	210	51	0	0	80.5	19.5
Kansas No. 6076:								
Abundant midlength	0	0	3	3	0	0	50.0	50.0
Few	0	57	205	18	0	20.4	73.2	6.4
Absent	0	3	107	141	0	1.2	42.6	56.2
Kansas No. 6090:								
Abundant midlength	0	221	5	0	0	97.8	2.2	0
Few	0	159	68	7	0	67.9	29.1	3.0
Absent	0	0	140	136	0	0	50.7	49.3
Kansas No. 6094:								
Abundant midlength	6	124	1	0	4.6	94.6	.8	0
Few	0	121	38	3	0	74.7	23.5	1.8
Absent	0	19	157	39	0	8.8	73.0	18.2
All strains:								
Abundant long	492	26	0	0	95.0	5.0	0	0
Abundant midlength	107	750	96	8	11.1	78.1	10.0	.8
Few	14	995	1,245	136	.6	41.6	52.1	5.7
Absent	8	105	1,202	1,733	.3	3.4	39.4	56.9
KANSAS AGRICULTURAL EXPERIMENT STATION								
Kansas No. 5020:								
Abundant midlength	179	50	141	0	48.4	13.5	38.1	0
Few	0	250	212	29	0	50.9	43.2	5.9
Absent	0	0	15	388	0	0	3.7	96.3
Kansas No. 5211:								
Abundant midlength	0	292	13	0	0	95.7	4.3	0
Few	0	297	40	0	0	88.1	11.9	0
Absent	0	2	235	558	0	.2	29.6	70.2
Kansas No. 5219:								
Abundant midlength	378	137	18	0	70.9	25.7	3.4	0
Few	0	1,088	440	26	0	70.0	28.3	1.7
Absent	0	41	478	599	0	3.7	42.7	53.6
Kansas No. 5220:								
Abundant midlength	0	36	39	2	0	46.8	50.6	2.6
Few	0	0	81	128	0	0	38.8	61.2
Absent	0	0	145	415	0	0	25.9	74.1
Kansas No. 6004:								
Absent	0	2	220	533	0	.3	29.1	70.6
Kansas No. 6052:								
Few	0	0	349	65	0	0	84.3	15.7
Absent	0	0	423	33	0	0	92.8	7.2
Kansas No. 6076:								
Absent	0	0	32	299	0	0	9.7	90.3
Kansas No. 6090:								
Abundant midlength	75	51	93	6	33.3	22.7	41.3	2.7
Few	0	184	196	24	0	45.5	48.5	5.9
Absent	0	0	71	282	0	0	20.1	79.9

TABLE VI.—*Data on inheritance of basal hairs in 9 strains of Burt oat grown at the Akron Field Station and at the Kansas Agricultural Experiment Station in 1921*—Continued

Strain number and basal hairs in parent kernels [a]	Basal hairs in progeny							
	Number of kernels with hairs—				Percentage of kernels with hairs—			
	Abundant		Few	Absent	Abundant		Few	Absent
	Long	Mid-length			Long	Mid-length		
KANSAS AGRICULTURAL EXPERIMENT STATION—continued								
Kansas No. 6094:								
Few	0	0	254	33	0	0	88.5	11.5
Absent	0	0	192	329	0	0	36.9	63.1
All strains:								
Abundant midlength	632	566	304	8	41.9	37.5	20.1	.5
Few	0	1,819	1,572	305	0	49.2	42.5	8.3
Absent	0	45	1,811	3,436	0	.9	34.2	64.9
BOTH STATIONS								
Abundant long	492	36	0	0	95.0	5.0	0	0
Abundant midlength	739	1,316	400	16	29.9	53.3	16.2	.6
Few	14	2,814	2,817	441	.2	46.2	46.3	7.3
Absent	8	150	3,013	5,169	.1	1.8	36.1	62.0

There is much variation in the number of twists and the length of the twisted portion. This type of awn is illustrated in Plate 3, A.

Of the nontwisted class, the awns in the long subclass often were fully as long as the twisted awns, but did not show the twisting of the lower portion or any geniculation. The nontwisted long awn is illustrated in Plate 3, B. The nontwisted short subclass was more variable than any other class. Awns placed in this subclass varied from a fairly well developed awn about 15 mm. in length down to very short bristlelike appendages, almost invisible to the naked eye. Kernels having the short awns are shown in Plate 3, C. When no awns were found, they were described as absent. Awnless kernels are shown in Plate 3, D.

The data on awns in the 1920 progeny are presented in Table VII. They indicate that all types of awns were found in the progeny of each parental awn class. As a rule, however, parental kernels in each awn class had a tendency to produce a greater number of progeny kernels of the parental class than of any of the other classes. Parental kernels having twisted awns produced progenies having twisted awns on about 56 per cent of the kernels, nontwisted long awns on 10 per cent, nontwisted short on 5 per cent, and awns absent on 30 per cent. In the progenies of the parental nontwisted class about 15 per cent of the awns were described as nontwisted long, 55 per cent as nontwisted short, 19 per cent absent, and about 11 per cent twisted. The parental kernels described as having awns absent produced progenies in which the awns of about 69 per cent of the kernels were described as absent, 13 per cent as nontwisted short, 8 per cent as nontwisted long, and 10 per cent as twisted.

An examination of the data for each of the strains given in Table VII shows that in four of the seven strains in which parental kernels having the twisted awns were found, from 50.5 to 86.3 per cent of the progeny kernels were described as having awns of that class. In Kansas No. 6004 only 21.2 per cent and in Kansas No. 6052 only 26.4 per cent of the progeny kernels grown from parental kernels with twisted awns were described as having twisted awns. As the two awn subclasses later described as nontwisted long and short were not separated in describing the parental kernels from which the 1920 crop was grown, the progenies representing these two subclasses may be considered together in discussing the results obtained in 1920. If this be done it is seen that in three of the strains the kernels having nontwisted awns produced rather high

TABLE VII.—*Data on inheritance of twisted, nontwisted (long and short), and absent awns in eight strains of Burt oat grown at the Akron Field Station in 1920*

Strain number and awn class in parent kernels[a]	Awn classes in progeny							
	Number of kernels with awns—				Percentage of kernels with awns—			
	Twisted	Nontwisted		Absent	Twisted	Nontwisted		Absent
		Long	Short			Long	Short	
Kansas No. 5020:								
Twisted	114	33	10	69	50.5	14.6	4.4	30.5
Nontwisted	107	158	562	233	10.1	14.9	53.0	22.0
Absent	166	158	194	1,157	9.9	9.4	11.6	69.1
Kansas No. 5211:								
Twisted	562	26	30	143	73.9	3.4	3.9	18.8
Nontwisted	458	815	3,602	661	8.3	14.7	65.1	11.9
Absent	389	289	557	2,105	11.6	8.7	16.7	63.0
Kansas No. 5220:								
Nontwisted	46	87	168	87	11.9	22.4	43.3	22.4
Absent	120	208	237	681	9.6	16.7	19.0	54.7
Kansas No. 6004:								
Twisted	18	12	26	29	21.2	14.1	30.6	34.1
Nontwisted	34	86	115	283	6.6	16.6	22.2	54.6
Absent	14	15	6	524	2.5	2.7	1.1	93.7
Kansas No. 6052:								
Twisted	138	82	31	271	26.4	15.7	5.9	51.9
Nontwisted	274	81	45	302	39.0	11.6	6.4	43.0
Absent	308	115	99	1,614	14.4	5.4	4.6	75.6
Kansas No. 6076:								
Twisted	141	75	14	138	38.3	20.4	3.8	37.5
Nontwisted	93	51	54	111	30.1	16.5	17.5	35.9
Absent	7	80	106	1,181	.5	5.8	7.7	86.0
Kansas No. 6090:								
Twisted	129	3	2	72	62.6	1.5	1.0	34.9
Nontwisted	53	324	1,077	303	3.0	18.4	61.3	17.3
Absent	74	87	294	1,150	4.6	5.4	18.3	71.7
Kansas No. 6094:								
Twisted	308	11	3	35	86.3	3.1	0.8	9.8
Nontwisted	163	137	654	179	14.4	12.1	57.7	15.8
Absent	211	88	187	597	19.5	8.1	17.3	55.1
All strains:								
Twisted	1,410	242	116	757	55.8	9.6	4.6	30.0
Nontwisted	1,228	1,739	6,377	2,159	10.7	15.1	55.4	18.8
Absent	1,289	1,040	1,682	9,009	9.9	8.0	12.9	69.2

[a] No differentiation was made between the long and the short nontwisted awns in describing the parent kernels.

percentages of each of the two extreme classes, namely, twisted and absent. In five of the eight strains parental kernels described as having nontwisted awns produced progeny of which the majority had the same type of awns.

Taking all strains together, the parental kernels described as having nontwisted awns produced 70.5 per cent of progeny kernels with nontwisted awns. The kernels described as having awns absent bred true to about as great an extent. In each of the eight strains over 50 per cent of the progeny of these kernels were awnless and the average for all strains was 69.2 per cent.

The data obtained in 1920 on the inheritance of awns indicate that parental kernels of the three classes reproduced their awn class in at least 50 per cent of their progeny. However, all classes of awns were produced in the progeny of each of the parental classes in all of the strains grown.

STUDY IN 1921

The data on the inheritance of awns in 1921 are presented in Table VIII. The twisted awn appears to breed comparatively true. Occasionally, probably for physiological reasons, kernels which carry the gene for twisted awns fail to produce any awns or awns as large as would be expected. In describing about 60,000 kernels of Burt oat the writers have observed that the twisted awn occasionally does not reach its full development until a rather late stage in the maturity of the plant and if unfavorable conditions prevent the normal maturing of the plant the development of the awn also is affected.

The awns described as nontwisted long apparently are unstable in breeding behavior, segregating into all classes ranging from twisted to absent. Parental kernels having nontwisted long awns produced about 74 per cent of such kernels in their progenies. The long and short subclasses grade into one another, making classification extremely difficult in many cases.

Parental kernels with nontwisted short awns produced 28.9 per cent of short-awned kernels and only 6.1 per cent of the twisted-awned kernels in their progenies, while parental kernels with the nontwisted long awns produced 7.2 and 14.9 per cent, respectively. From this it appears that these two subclasses, that is, the long and short awned, are somewhat different genetically. Kernels with awns absent reproduced that character in 59.3 per cent of the progenies, while 4.4 and 20.9 per cent, respectively, were nontwisted short and nontwisted long and 15.4 per cent were twisted.

In general the results on the inheritance of awns obtained by the writers correspond with those of previous investigators, except that previous workers have not differentiated between nontwisted long and short awns. In the Burt oat the absent or awnless condition seems partially dominant, while the twisted awn seems recessive. The influence of physiological and other factors may account for the fact that some kernels which apparently carry the gene for producing awns failed to produce them. The possible influence of environmental factors on the development of awns in oats has been recognized by several other investigators.

Awns are readily observable and have been studied by more investigators than have some of the other characters of the oat spikelet. Nilsson-Ehle (*95*) states that certain yellow oats carry an inhibitor for awns. Fraser (*37*) in studying the cross Burt × Sixty-Day found no evidence of the existence of such an inhibiting factor in the Burt variety, but his ideas were in agreement with those of Nilsson-Ehle with respect to the presence of an awn-inhibiting factor in yellow oat kernels in the Sixty-Day variety. In the crossing experiments of Zade (*153*), Nilsson-Ehle (*91, 93*), Surface (*127*), Love and Craig (*70*), Love and Fraser (*69*), and Fraser (*37*), it was found as a rule that in crosses of awned × awnless oats the F_1 was intermediate and in F_2 the fully awned type behaved as a recessive. Fraser (*37*) reports that in the cross of Burt, which he states was usually awned, and Sixty-Day awnless, he found nearly complete dominance of the awnless type in F_1.

LEMMA COLOR

The color of the oat lemma probably is the most complex of all of the characters considered. This is due in part to its being much more easily affected by physiological and climatic conditions than most of the other characters, which are morphological. In its genetic basis also lemma color in the Burt oat is certainly very complex, perhaps more so than any of the other spikelet characters studied. The color of the lemma is not fully developed until the kernel approaches maturity. Hence any condition which interferes with normal ripening has a marked effect on the color and makes it one of the most difficult oat characters to classify accurately. For these reasons it may be considered less important from the genetic and taxonomic standpoint, although it is perhaps the character with which agronomists, grain inspectors, seedsmen, and farmers are most familiar.

The lemma color in the progenies grown was described as black, dark brown, light brown, red, yellow, and white. An incomplete series of kernel colors is shown in Plates 4 to 6, inclusive. The names of the color classes used in the text and in the tables are those commonly recognized by agronomists. The colors graded into one another, and often it was extremely difficult to determine the correct color classification. This was especially true of the lighter colored kernels.

STUDY IN 1920

In the classification of the parental material at Akron in 1920 a class termed "variegated" was created for those kernels showing a more or less mottled color. Very few variegated kernels were found in the progenies harvested in 1920, apparently proving that variegation resulted from climatic conditions or physiological causes. For this reason the variegated class was no longer used. Data were obtained in only two of the eight strains on the 1920 progeny kernels from parental kernels described as variegated. In describing the original material no distinction was made at either station between the dark and light shades of brown. In describing the 1920 progenies from the Manhattan station and one strain, Kansas No. 5220, from the Akron station, no distinction was made between the two shades of brown. This differentiation was made in the other seven strains grown at Akron in 1920.

A few grayish-brown kernels were observed in the course of the experiments, but as they were not of frequent

TABLE VIII.—*Data on inheritance of twisted, nontwisted (long and short), and absent awns in 9 strains of Burt oat grown at the Akron Field Station and at the Kansas Agricultural Experiment Station in 1921*

Strain number and awn class in parent kernels	Awn classes in progeny							
	Number of kernels with awns—				Percentage of kernels with awns—			
	Twisted	Nontwisted		Absent	Twisted	Nontwisted		Absent
		Long	Short			Long	Short	
AKRON FIELD STATION								
Kansas No. 5020:								
Twisted	91	20	11	19	64.5	14.2	7.8	13.5
Nontwisted long	52	63	28	16	32.7	39.6	17.6	10.1
Nontwisted short	23	130	70	20	9.5	53.5	28.8	8.2
Absent	15	95	44	355	2.9	18.7	8.6	69.7
Kansas No. 5211:								
Twisted	384	15	0	10	93.9	3.7	0	2.4
Nontwisted long	44	49	7	8	40.7	45.4	6.5	7.4
Nontwisted short	70	822	372	142	5.0	58.5	26.4	10.1
Absent	198	249	40	741	16.1	20.3	3.3	60.3
Kansas No. 5220:								
Twisted	5	1	0	1	71.4	14.3	0	14.3
Nontwisted long	34	42	7	24	31.8	39.3	6.5	22.4
Nontwisted short	13	83	37	29	8.0	51.2	22.9	17.9
Absent	13	39	10	228	4.5	13.4	3.5	78.6
Kansas No. 6004:								
Absent	7	2	1	19	24.1	6.9	3.5	65.5
Kansas No. 6052:								
Nontwisted short	29	33	3	12	37.7	42.8	3.9	15.6
Absent	28	12	10	211	10.7	4.6	3.8	80.9
Kansas No. 6076:								
Twisted	83	2	0	3	94.3	2.3	0	3.4
Nontwisted long	0	58	23	0	0	71.6	28.4	0
Nontwisted short	0	42	75	2	0	35.3	63.0	1.7
Absent	67	25	16	141	26.9	10.1	6.4	56.6
Kansas No. 6090:								
Twisted	63	0	0	2	96.9	0	0	3.1
Nontwisted long	0	50	30	0	0	62.5	37.5	0
Nontwisted short	3	173	157	43	.8	46.0	41.8	11.4
Absent	3	23	21	168	1.4	10.7	9.8	78.1
Kansas No. 6094:								
Twisted	47	0	1	2	94.0	0	2.0	4.0
Nontwisted short	1	87	37	6	.8	66.4	28.2	4.6
Absent	40	163	41	83	12.2	49.9	12.5	25.4
All strains:								
Twisted	673	38	12	37	88.5	5.0	1.6	4.9
Nontwisted long	130	262	95	48	24.3	49.0	17.7	9.0
Nontwisted short	139	1,370	751	254	5.5	54.5	29.9	10.1
Absent	371	608	183	1,946	11.9	19.6	5.9	62.6
KANSAS AGRICULTURAL EXPERIMENT STATION								
Kansas No. 5020:								
Twisted	200	0	0	0	100.0	0	0	0
Nontwisted long	3	0	0	0	100.0	0	0	0
Nontwisted short	0	105	76	2	0	57.4	41.5	1.1
Absent	26	101	12	311	5.8	22.4	2.7	69.1
Kansas No. 5211:								
Twisted	654	148	0	9	80.6	18.3	0	1.1
Nontwisted long	53	217	7	5	18.8	76.9	2.5	1.8
Kansas No. 5219:								
Twisted	283	26	5	36	80.9	7.4	1.4	10.3
Nontwisted long	204	911	75	13	17.0	75.7	6.2	1.1
Nontwisted short	40	152	26	34	15.9	60.3	10.3	13.5
Absent	165	127	14	167	34.9	26.8	3.0	35.3
Kansas No. 5220:								
Twisted	119	50	2	6	67.2	28.3	1.1	3.4
Nontwisted long	32	278	11	24	9.3	80.6	3.2	6.9
Absent	68	113	3	8	35.4	58.8	1.6	4.2
Kansas No. 6004:								
Twisted	260	130	3	109	51.8	25.9	.6	21.7
Nontwisted long	30	45	1	2	38.4	57.7	1.3	2.6
Absent	3	59	0	91	1.9	38.6	0	59.5

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TABLE VIII.—*Data on inheritance of twisted, nontwisted (long and short), and absent awns in 9 strains of Burt oat grown at the Akron Field Station and at the Kansas Agricultural Experiment Station in 1921*—Continued

Strain number and awn class in parent kernels	Awn classes in progeny							
	Number of kernels with awns—				Percentage of kernels with awns—			
	Twisted	Nontwisted		Absent	Twisted	Nontwisted		Absent
		Long	Short			Long	Short	
KANSAS AGRICULTURAL EXPERIMENT STATION—continued								
Kansas No. 6052:								
Twisted	692	45	4	32	89.5	5.8	0.5	4.2
Absent	115	5	2	6	89.8	3.9	1.6	4.7
Kansas No. 6076:								
Twisted	153	0	0	0	100.0	0	0	0
Kansas No. 6090:								
Nontwisted long	1	396	31	22	.2	88.0	6.9	4.9
Absent	0	4	0	349	0	1.1	0	98.9
Kansas No. 6094:								
Twisted	602	60	0	0	90.9	9.1	0	0
Nontwisted long	2	160	1	0	1.2	98.2	.6	0
All strains:								
Twisted	2,963	459	14	192	81.7	12.6	.4	5.3
Nontwisted long	325	2,007	126	66	12.9	79.5	5.0	2.6
Nontwisted short	40	257	102	36	9.2	59.1	23.4	8.3
Absent	377	409	31	932	21.6	23.4	1.8	53.3
BOTH STATIONS								
Twisted	3,636	497	26	229	82.9	11.3	.6	5.2
Nontwisted long	455	2,269	221	114	14.9	74.2	7.2	3.7
Nontwisted short	179	1,627	853	290	6.1	55.2	28.9	9.8
Absent	748	1,017	214	2,878	15.4	20.9	4.4	59.3

occurrence and could not always be clearly distinguished from the kernels classed as light brown, no separate classification was made for them. It is possible that some of the kernels included in the variegated and red classes should have been classed as gray. Kernels having a grayish-brown color are shown in Plate 6, P.

The data on the inheritance of kernel color are presented in Table IX. In general, the dark-colored kernels produced mostly dark-colored progeny, while the light-colored kernels tended to produce light-colored kernels in their progenies. The black parental kernels produced mostly dark-brown and black progeny kernels and a comparatively small percentage of light brown and red kernels. Brown kernels largely produced brown kernels of different shades and also about 26 per cent of reds and a few yellows. Red parental kernels produced about 69 per cent of red kernels in their progenies. Yellow kernels produced about 81 per cent of red kernels, and white parental kernels produced mostly red and yellow kernels in their progenies. While none of the colors appeared constant in breeding behavior, there was a rather definite relation between the color of the parental and progeny kernels.

EXPLANATORY LEGEND FOR PLATE 6

Lemma colors of 7 strains of Burt oat in 1920. A to C.—Kansas No. 5219: A, very dark brown, yellow tipped; B, reddish brown; C, yellowish white. D.—Kansas No. 6094, light brown. E.—Kansas No. 5020, light yellowish brown. F to G.—Kansas No. 5211: F, light yellowish brown; G, light brownish yellow. H to N.—Kansas No. 5020: H, very dark gray; I, dark gray; J, grayish yellow; K, grayish white; L, light grayish yellow; M, dark grayish brown; N, grayish white. O.—Kansas No. 6076, grayish yellow. P.—Kansas No. 5020, grayish brown, streaked. Q.—Kansas No. 5211, light brownish yellow. R.—Kansas No. 6052, yellowish white. S.—Kansas No. 6094, light grayish yellow. T.—Kansas No. 6052, dark grayish brown. U.—Kansas No. 6090, light brown. V.—Kansas No. 5219, dark brown. W.—Kansas No. 5211, light brown. X.—Kansas No. 6052, light yellowish white. Y.—Kansas No. 5219, very dark brown.

TABLE IX.—*Data on inheritance of lemma color in 9 strains of Burt oat grown at the Akron Field Station and at the Kansas Agricultural Experiment Station in 1920*

Strain number and color of parent kernels	Color of lemmas in lower florets of progeny													
	Number of lemmas—							Percentage of lemmas—						
	Black	Dark brown	Brown[a]	Light brown	Red	Yellow	White	Black	Dark brown	Brown[a]	Light brown	Red	Yellow	White
AKRON FIELD STATION														
Kansas No. 5020:														
Black	67	189	.....	0	0	0	0	26.2	73.8	.....	0	0	0	0
Brown	15	177	.....	170	170	0	0	2.8	33.2	.....	32.0	32.0	0	0
Red	0	28	.....	62	929	15	0	0	2.7	.....	6.0	89.8	1.5	0
Yellow	0	0	.....	0	639	17	0	0	0	.....	0	97.4	2.6	0
White	0	0	.....	0	460	23	0	0	0	.....	0	95.2	4.8	0
Kansas No. 5211:														
Black	89	555	.....	0	32	0	0	13.2	82.1	.....	0	4.7	0	0
Brown	90	571	.....	180	259	0	0	8.2	51.9	.....	16.4	23.5	0	0
Red	0	75	.....	233	2,281	179	0	0	2.7	.....	8.4	82.4	6.5	0
Yellow	0	0	.....	138	2,108	349	37	0	0	.....	5.2	80.1	13.3	1.4
White	0	0	.....	11	736	849	188	0	0	.....	.6	41.3	47.6	10.5
Variegated[b]	0	0	.....	1	673	103	0	0	0	.....	.1	86.6	13.3	0
Kansas No. 5220:														
Black	100	0	129	0	0	0	0	43.7	0	56.3	0	0	0	0
Brown	42	0	114	0	0	0	0	26.9	0	73.1	0	0	0	0
Red	0	0	85	0	348	452	0	0	0	9.6	0	39.3	51.1	0
Yellow	0	0	13	0	120	152	0	0	0	4.6	0	42.1	53.3	0
White	20	0	30	0	1	3	25	25.3	0	38.0	0	1.3	3.8	31.6
Kansas No. 6004:														
Brown	0	0	.....	0	192	0	0	0	0	.....	0	100.0	0	0
Red	0	0	.....	0	410	23	0	0	0	.....	0	94.7	5.3	0
Yellow	0	0	.....	0	374	17	0	0	0	.....	0	95.7	4.3	0
White	0	0	.....	0	143	5	0	0	0	.....	0	96.6	3.4	0
Kansas No. 6052:														
Black	35	44	.....	0	0	0	0	44.3	55.7	.....	0	0	0	0
Brown	0	93	.....	27	119	41	0	0	33.2	.....	9.6	42.5	14.6	0
Red	0	0	.....	0	219	120	1	0	0	.....	0	64.4	35.3	.3
Yellow	0	0	.....	1	1,841	146	0	0	0	.....	.1	92.6	7.3	0
White	0	0	.....	0	392	271	10	0	0	.....	0	58.2	40.3	1.5
Kansas No. 6076:														
Black	17	41	.....	10	0	0	0	25.0	60.3	.....	14.7	0	0	0
Brown	18	41	.....	17	266	0	0	5.3	12.0	.....	4.9	77.8	0	0
Red	0	0	.....	0	438	2	0	0	0	.....	0	99.5	.5	0
Yellow	0	0	.....	0	453	27	0	0	0	.....	0	94.4	5.6	0
White	0	0	.....	0	596	122	3	0	0	.....	0	82.7	16.9	.4

Kansas No. 6090:														
Black	43	633		23	101	0	0	5.4	79.1		2.9	12.6	0	0
Brown	12	1,105		78	131	9	0	.9	82.8		5.8	9.8	.7	0
Red	0	316		89	375	0	0	0	40.5		11.4	48.1	0	0
Yellow	0	8		59	245	36	0	0	2.3		17.0	70.4	10.3	0
White	0	154		6	135	10	0	0	50.5		2.0	44.2	3.3	0
Kansas No. 6094:														
Black	0	220		3	25	0	0	0	88.7		1.2	10.1	0	0
Brown	0	229		0	154	1	0	0	59.6		0	40.1	.3	0
Red	0	25		0	590	22	0	0	3.9		0	92.6	3.5	0
Yellow	0	0		0	626	71	23	0	0		0	86.9	9.9	3.2
White	0	0		0	440	94	0	0	0		0	82.4	17.6	0
Variegated [b]	0	25		0	25	0	0	0	50.0		0	50.0	0	0
All strains:														
Black	351	1,682	129	36	158	0	0	14.9	71.4	5.5	1.5	6.7	0	0
Brown [a]	177	2,216	114	472	1,291	51	0	4.1	51.3	2.6	10.9	29.9	1.2	0
Red	0	444	85	384	5,590	813	1	0	6.1	1.2	5.2	76.4	11.1	0
Yellow	0	8	13	198	6,406	815	60	0	.1	.2	2.6	85.4	10.9	.8
White	20	154	30	17	2,903	1,377	226	.4	3.3	.6	.4	61.4	29.1	4.8
Variegated [b]	0	25	0	1	698	103	0	0	3.0	0	.1	84.4	12.5	0

KANSAS AGRICULTURAL EXPERIMENT STATION

Kansas No. 5020:														
Brown	27		644		81	0	0	3.6		85.6		10.8	0	0
Red	0		155		79	14	3	0		61.7		31.5	5.6	1.2
Yellow	0		94		216	13	0	0		29.1		66.9	4.0	0
White	0		101		168	45	3	0		31.9		53.0	14.2	.9
Kansas No. 5211:														
Black	0		33		27	0	0	0		55.0		45.0	0	0
Brown	50		755		475	29	0	3.8		57.7		36.3	2.2	0
Yellow	0		80		297	39	0	0		19.2		71.4	9.4	0
White	0		86		115	73	0	0		31.4		42.0	26.6	0
Kansas No. 5219:														
Black	4		20		0	0	0	16.7		83.3		0	0	0
Brown	33		502		182	1	0	4.6		69.9		25.4	.1	0
Red	0		152		128	9	0	0		52.6		44.3	3.1	0
White	3		576		241	53	0	.3		66.0		27.6	6.1	0
Kansas No. 5220:														
Black	4		13		1	0	0	22.2		72.2		5.6	0	0
Brown	9		294		223	90	1	1.5		47.6		36.1	14.6	.2
Red	0		12		90	34	0	0		8.8		66.2	25.0	0
Yellow	0		0		75	6	0	0		0		92.6	7.4	0
White	0		0		22	1	4	0		0		81.5	3.7	14.8
Kansas No. 6004:														
Yellow	0		69		366	28	0	0		14.9		79.0	6.1	0
White	0		179		217	78	0	0		37.8		45.8	16.4	0

[a] In 1 strain, Kansas No. 5220, grown at Akron and in all 9 strains grown at Manhattan in 1920, all brown lemmas were recorded as brown, instead of being separated into dark brown and light brown as was done in describing the other 7 strains grown at Akron.

[b] In only 2 strains, Kansas Nos. 5211 and 6094, were the progeny kernels from parental kernels classed as "variegated" included at Akron.

TABLE IX.—*Data on inheritance of lemma color in 9 strains of Burt oat grown at the Akron Field Station and at the Kansas Agricultural Experiment Station in 1920*—Continued

Strain number and color of parent kernels	Color of lemmas in lower florets of progeny													
	Number of lemmas—							Percentage of lemmas—						
	Black	Dark brown	Brown[a]	Light brown	Red	Yellow	White	Black	Dark brown	Brown[a]	Light brown	Red	Yellow	White
KANSAS AGRICULTURAL EXPERIMENT STATION—continued.														
Kansas No. 6052:														
Brown	0		26		8	0	0	0		76.5		23.5	0	0
Red	0		285		469	348	16	0		25.5		42.0	31.1	1.4
Yellow	0		12		40	22	0	0		16.2		54.1	29.7	0
White	0		65		88	42	0	0		33.3		45.1	21.6	0
Kansas No. 6076:														
Brown	0		62		0	0	0	0		100.0		0	0	0
Red	11		60		18	0	0	12.4		67.4		20.2	0	0
Yellow	0		46		139	189	0	0		12.3		37.2	50.5	0
White	0		25		156	289	0	0		5.3		33.2	61.5	0
Kansas No. 6090:														
Black	3		47		0	0	0	6.0		94.0		0	0	0
Brown	32		952		51	11	0	3.1		91.0		4.9	1.0	0
Red	0		24		15	0	0	0		61.5		38.5	0	0
Yellow	0		76		30	0	0	0		71.7		28.3	0	0
White	0		88		32	0	0	0		73.3		26.7	0	0
Kansas No. 6094:														
Black	0		21		0	0	0	0		100.0		0	0	0
Brown	2		240		75	14	0	.6		72.5		22.7	4.2	0
Red	0		50		150	65	5	0		18.5		55.6	24.1	1.8
Yellow	0		0		0	32	0	0		0		0	100.0	0
White	0		28		100	101	0	0		12.2		43.7	44.1	0
All strains:														
Black	11		134		28	0	0	6.3		77.5		16.2	0	0
Brown	153		3,475		1,095	145	1	3.1		71.4		22.5	3.0	0
Red	11		738		949	470	24	.5		33.7		43.3	21.4	1.1
Yellow	0		377		1,163	329	0	0		20.2		62.2	17.6	0
White	3		1,148		1,139	682	7	.1		38.6		38.2	22.9	.2
BOTH STATIONS														
Black	362	1,682	263	36	186	0	0	14.3	66.5	10.4	1.4	7.4	0	0
Brown	330	2,216	3,589	472	2,386	196	1	3.6	24.1	39.1	5.1	26.0	2.1	0
Red	11	444	823	384	6,539	1,283	25	.1	4.7	8.6	4.0	68.8	13.5	.3
Yellow	0	8	390	198	7,569	1,144	60	0	.1	4.2	2.1	80.8	12.2	.6
White	23	154	1,178	17	4,042	2,059	233	.3	2.0	15.3	.2	52.5	26.7	3.0
Variegated[b]	0	25	0	1	698	103	0	0	3.0	0	.1	84.4	12.5	0

[a] In 1 strain, Kansas No. 5220, grown at Akron and in all 9 strains grown at Manhattan in 1920, all brown lemmas were recorded as brown, instead of being separated into dark brown and light brown as was done in describing the other 7 strains grown at Akron.

[b] In only 2 strains, Kansas Nos. 5211 and 6094, were the progeny kernels from parental kernels classed as "variegated" included at Akron.

STUDY IN 1921

Because of the injury caused by chinch bugs at Manhattan no data on kernel color are presented for the crop grown there in 1921. The data from Akron, given in Table X, indicate that some of the kernels classed as black and dark brown probably are similar in breeding behavior. The color described as dark brown in many cases probably is the result of the imperfect development of black. The results obtained in 1921 are similar to those of 1920 in that the dark-colored parental kernels show a very strong tendency to produce dark-colored kernels in their progenies.

The light brown color class probably contains many genetic reds, which, due to physiological influences, appear as light brown. The fact that the parental kernels described as light brown produced about 71 per cent of red kernels in their progenies supports this statement. Red was the most stable of the kernel colors. Red kernels produced only small percentages of other colors in their progenies. A few light brown and yellow kernels were produced in the progenies of red kernels, which may have been due in part to the effect of physiological factors, which favor an intensification in the one case and a dilution or incomplete development of red color in the other. Many parental kernels which were described as yellow apparently were genetic reds, in which the red color for some reason did not attain its normal development.

Comparatively few yellows bred true, most of them producing reds of varying intensities, and including occasional kernels described as light brown. Kernels classified as white appear to be of two classes, those which for physiological reasons did not fully develop their normal color and thus appeared white, although genetically really colored, and those which were genetically white. Comparatively few (26.2 per cent) of the kernels in the progenies of parental white kernels were described as white. It appears difficult to obtain strains of Burt oat which will breed true for white kernel color. This is not an unexpected condition when it is remembered that this variety is known to contain factors for black, several shades of brown, red, yellow, and probably gray kernel colors. It may be supposed that all of these factors would have to be absent or in the recessive condition to permit the production of homozygous white kernels.

The occurrence of dark-colored gray and brown kernels in the cross Burt (red) × Sixty-Day (yellow) is explained by Fraser (*37*) as probably due to reversion or to mutation. The results obtained in these experiments clearly indicate that the dark-colored kernels which occur in Burt oat are the result of genetic factors for dark color, carried by many strains in this variety. The advisability of assigning only two color factors for the variety Burt, as Fraser has done, is questioned, though probably he meant only to indicate that these were the particular factors considered in his experiments and not necessarily the only ones present in the variety. Further and more carefully controlled experiments are needed, for as Fraser has stated—

> Considerable variation in color is to be noted even within the same pure line during different seasons, or under strikingly different environmental conditions.

The results obtained in these experiments indicate that the Burt oat not only contains the "R" and "Y" color factors mentioned by Fraser (*37*) in the strain with which he worked but probably several additional color factors. The strain of Burt used by Fraser doubtless was a light-colored form. Most of the strains of Burt oat observed by the writers have had at least a few dark-colored kernels, including blacks or dark browns, light browns, and grays, which produced dark-colored kernels in their progenies.

ASSOCIATION OF SPIKELET CHARACTERS

An attempt was made to determine what correlation, if any, existed between the kernel characters studied. These studies were made from the original descriptions of the 1920 crop grown at Manhattan. No correlation data are presented on the material grown at Akron in 1920 nor on the material grown at either station in 1921. Studies of correlation or association were made of the following pairs of characters: (1) Floret disjunction and spikelet disarticulation, (2) spikelet disarticulation and basal hairs, (3) spikelet disarticulation and awns, (4) lemma color and spikelet disarticulation, (5) lemma color and awns.

These studies indicate very clearly that certain spikelet and floret characters "tend to go together in heredity," and that genetic coupling or linkage probably is involved. It has been thought best, however, to use the terms association and correlation in the discussion of these data, for

TABLE X.—*Data on inheritance of lemma color in 8 strains of Burt oat grown at the Akron Field Station in 1921*

Strain number and color of parent lemmas	Color of lemmas in lower florets of progeny											
	Number of lemmas—						Percentage of lemmas—					
	Black	Dark brown	Light brown	Red	Yellow	White	Black	Dark brown	Light brown	Red	Yellow	White
Kansas No. 5020:												
Black	6	154	29				3.2	81.5	15.3			
Dark brown		106	173	33				34.0	55.4	10.6		
Light brown				163						100.0		
Red			4	193	40				1.7	81.4	16.9	
Yellow				130	21					86.1	13.9	
Kansas No. 5211:												
Black			76	30					71.7	28.3		
Dark brown	60	126	104	18			19.5	40.9	33.8	5.8		
Light brown				216						100.0		
Red				641	4					99.4	.6	
Yellow			16	987	209	5			1.3	81.1	17.2	0.4
White				157	255	182				26.4	42.9	30.7
Kansas No. 5220:												
Black		9	56	13				11.5	71.8	16.7		
Light brown			101	61					62.3	37.7		
Red				39						100.0		
Yellow				227	31					88.0	12.0	
White				9	2					81.8	18.2	
Kansas No. 6004:												
Light brown				16						100.0		
Yellow					13						100.0	
Kansas No. 6052:												
Black		4	38	34	1			5.2	49.3	44.2	1.3	
Dark brown		18	151	39				8.7	72.6	18.7		
Red				22	31					41.5	58.5	
Kansas No. 6076:												
Black		123	12	8				86.0	8.4	5.6		
Dark brown		33	46					41.8	58.2			
Red		3		264	8			1.1		96.0	2.9	
Kansas No. 6090:												
Black	19	47	45				17.1	42.4	40.5			
Dark brown	4	11	49	78	3		2.7	7.6	33.8	53.8	2.1	
Light brown		8	83	12				7.8	80.6	11.6		
Red			24	278					7.9	92.1		
Yellow				71						100.0		
Kansas No. 6094:												
Dark brown		13	93	33				9.4	66.9	23.7		
Red			8	117	1				6.3	92.9	.8	
Yellow				109	16					87.2	12.8	
White				91						100.0		
SUMMARY												
All strains:												
Black	25	337	256	83	1		3.6	48.0	36.5	11.8	.1	
Dark brown	64	307	616	201	3		5.4	25.8	51.7	16.9	.2	
Red		8	184	468				1.2	27.9	70.9		
Yellow		3	36	1,554	84			.2	2.1	92.7	5.0	
White			16	1,524	290	5			.9	83.0	15.8	.3
				257	257	182				36.9	36.9	26.2

the reason that they were not obtained from controlled crosses of parents of known factorial composition for the characters under observation, the conditions usually considered essential for the accurate study of linkage relations. The data on association of kernel characters are presented in Tables XI to XV in such a way that both actual or observed numbers and percentages for each pair of characters can be seen at a glance.

In addition, the coefficient of association (Q) of Yule (*152*) has been determined for each of the five association groups. In order to use the formula,

$$Q=\frac{ad-bc}{ad+bc},$$

it was necessary to group the data for any character into two contrasting classes. Thus, in determining association between awns and kernel color, the kernels were classed as awned or

TABLE XI.—*Data on association of class of floret disjunction and spikelet disarticulation in 9 strains of Burt oat grown at the Kansas Agricultural Experiment Station in 1920*

Strain number and kind of floret disjunction	Spikelet disarticulation					
	Number of kernels disarticulating by—			Percentage of kernels disarticulating by—		
	Abscission	Semiabscission	Fracture	Abscission	Semiabscission	Fracture
Kansas No. 5020:						
Basifracture	75	88	105	28.0	32.8	39.2
Heterofracture	30	16	39	35.3	18.8	45.9
Disarticulation	104	292	894	8.1	22.6	69.3
Kansas No. 5211:						
Basifracture	312	124	175	51.1	20.3	28.6
Heterofracture	114	48	51	53.5	22.5	24.0
Disarticulation	373	260	602	30.2	21.1	48.7
Kansas No. 5219:						
Basifracture	325	302	87	45.5	42.3	12.2
Heterofracture	89	62	25	50.6	35.2	14.2
Disarticulation	323	404	287	31.9	39.8	28.3
Kansas No. 5220:						
Basifracture	41	31	58	31.5	23.9	44.6
Heterofracture	22	8	37	32.8	12.0	55.2
Disarticulation	38	130	514	5.6	19.0	75.4
Kansas No. 6004:						
Basifracture	0	5	63	0	7.4	92.6
Heterofracture	0	1	19	0	5.0	95.0
Disarticulation	0	12	837	0	1.4	98.6
Kansas No. 6052:						
Basifracture	0	2	74	0	2.6	97.4
Heterofracture	0	0	34	0	0	100.0
Disarticulation	0	19	1,292	0	1.4	98.6
Kansas No. 6076:						
Basifracture	0	27	62	0	30.3	69.7
Heterofracture	0	5	7	0	41.7	58.3
Disarticulation	0	58	836	0	6.5	93.5
Kansas No. 6090:						
Basifracture	162	102	49	51.8	32.6	15.6
Heterofracture	32	30	18	40.0	37.5	22.5
Disarticulation	153	275	540	15.8	28.4	55.8
Kansas No. 6094:						
Basifracture	52	54	46	34.2	35.5	30.3
Heterofracture	11	9	7	40.8	33.3	25.9
Disarticulation	115	208	381	16.3	29.6	54.1
All strains:						
Basifracture	967	735	719	39.9	30.4	29.7
Heterofracture	298	179	237	41.7	25.1	33.2
Disarticulation	1,106	1,658	6,183	12.4	18.5	69.1

$Q=0.672\pm0.003$

awnless, on the one hand, and as dark or light colored on the other. It is true, of course, that the coefficient of association based on this grouping gives no information as to the relations between the individual phases of the two characters. However, it is believed that the coefficient of association determined in this manner does give a reliable index of the degree of association which exists between the two characters. This method has been proposed and used in somewhat similar though not exactly comparable cases by Collins (*21*) and later by Kempton (*57*). By this method the complete independence of two character pairs is represented by 0 and complete association by 1. Intermediate degrees of relationship are expressed by the intermediate decimals.

The probable errors of the coefficients of association as given in the text and in Tables XI to XV were determined by substituting Q for r in the following formula:

$$E_r=\frac{0.6745\ (1-r^2)}{\sqrt{n}},$$

which is the one generally used for computing probable errors of correlation coefficients. While the values thus obtained are admittedly only approximations, it is believed that they do not deviate from the true values for the probable errors of Q any more than, if as much as, the values for Q itself differ from the values for r. The relations of these two constants are shown in the following table from Yule (*152*).

Corresponding values of Q and r

Q	r	Q	r
0.0	0.000	0.5	0.409
.1	.079	.6	.500
.2	.158	.7	.598
.3	.239	.8	.707
.4	.322	.9	.833
		1.0	1.000

Yule states that for corresponding values of Q and r the probable error of Q is less, not greater, than that of r, that is, if we form Q and r for the same material the probable error of the former constant is the smaller. The ratio of the standard error of Q to the standard error of r is very close, as shown in the following table taken from Yule (*152*):

Ratio of the standard error of Q to the standard error of r for equal numerical values of Q and r

Q	Ratio	Q	Ratio
0.1	1.001	0.6	1.061
.2	1.005	.7	1.095
.3	1.012	.8	1.155
.4	1.023	.9	1.283
.5	1.038	1.0	1.000

FLORET DISJUNCTION AND SPIKELET DISARTICULATION

Table XI presents the summarized data obtained in the study of the relation between floret disjunction and spikelet disarticulation. These data show clearly that some relation exists between these characters, although it is less pronounced in some of the strains than in others.

The basifracture type of floret disjunction and the abscission type of spikelet disarticulation might naturally be expected to be correlated. Similarly floret disjunction by disarticulation and spikelet disarticulation by fracture might be expected to be closely associated because these combinations are typical of the two species. If progenies of the heterofracture floret disjunction are not included, the results are as follows:

Floret disjunction by—	Spikelet disarticulation by—		
	Abscission	Semiabscission	Fracture
	Per cent	*Per cent*	*Per cent*
Basifracture	39.9	30.4	29.7
Disarticulation	12.4	18.5	69.1

Apparently the correlation between floret disjunction by disarticulation and spikelet disarticulation by fracture as a rule is more pronounced than that between floret disjunction by basifracture and spikelet disarticulation by abscission. The coefficient of association (Q) in this case was determined by adding the number of kernels in which floret disjunction was by heterofracture to that by basifracture and by adding those in which spikelet disarticulation was from abscission and semiabscission respectively. Arranged in this way, the data are as follows:

Floret disjunction by—	Spikelet disarticulation by—	
	Abscission and semiabscission	Fracture
Basifracture, Heterofracture	2,179	956
Disarticulation	2,764	6,183

$Q = 0.672 \pm 0.003$

These data indicate that a high degree of association exists ($Q = 0.672 \pm 0.003$) between certain phases of spikelet disarticulation and certain phases of floret disjunction. Spikelet disarticulation resulting from abscission and semiabscission apparently is closely associated with floret disjunction by basifracture and heterofracture, while spikelet disarticulation by fracture is associated with floret disjunction by disarticulation. In other words, the two *byzantina* characters are associated as also are the two *sativa* characters.

SPIKELET DISARTICULATION AND BASAL HAIRS

The data on correlation between spikelet disarticulation and basal hairs are presnted in Table XII. It is evident that correlation exists between spikelet disarticulation by fracture (without a basal cavity) and the absence of basal hairs. Correlation exists between the basal cavity (resulting from abscission) and the presence of basal hairs, but this correlation does not seem to be as marked as the former one. In determining the value of Q for these characters, the kernels disarticulating by semiabscission were added to those disarticulating by abscission and the abundant long, abundant midlength, and few classes of basal hairs were all combined into one class and considered as basal hairs present, the data being arranged as follows:

Spikelet disarticulation by—	Basal hairs	
	Present	Absent
Abscission, Semiabscission	4,354	589
Fracture	1,855	5,284

$Q = 0.909 \pm 0.001$

TABLE XII.—*Data on association of spikelet disarticulation and basal hairs in 9 strains of Burt oat grown at the Kansas Agricultural Experiment Station in 1920*

Strain number and kind of spikelet disarticulation in kernels	Basal hairs							
	Number of kernels having hairs—				Percentage of kernels having hairs—			
	Abundant		Few	Absent	Abundant		Few	Absent
	Long	Mid-length			Long	Mid-length		
Kansas No. 5020:								
Abscission	0	111	95	3	0	53.1	45.5	1.4
Semiabscission	0	18	336	42	0	4.5	84.9	10.6
Fracture	0	0	282	756	0	0	27.2	72.8
Kansas No. 5211:								
Abscission	60	247	481	11	7.5	30.9	60.2	1.4
Semiabscission	0	25	361	46	0	5.8	83.6	10.6
Fracture	0	0	154	674	0	0	18.6	81.4
Kansas No. 5219:								
Abscission	27	122	537	51	3.6	16.6	72.9	6.9
Semiabscission	2	78	612	76	.3	10.1	79.7	9.9
Fracture	0	0	63	336	0	0	15.8	84.2
Kansas No. 5220:								
Abscission	0	18	80	3	0	17.8	79.2	3.0
Semiabscission	0	14	93	62	0	8.3	55.0	36.7
Fracture	0	0	153	456	0	0	25.1	74.9
Kansas No. 6004:								
Semiabscission	0	0	17	1	0	0	94.4	5.6
Fracture	0	0	348	571	0	0	37.9	62.1
Kansas No. 6052:								
Semiabscission	0	0	16	5	0	0	76.2	23.8
Fracture	0	0	286	1,114	0	0	20.4	79.6
Kansas No. 6076:								
Semiabscission	0	0	39	51	0	0	43.3	56.7
Fracture	0	0	370	535	0	0	40.9	59.1
Kansas No. 6090:								
Abscission	0	108	219	20	0	31.1	63.1	5.8
Semiabscission	0	23	252	132	0	5.7	61.9	32.4
Fracture	0	0	94	513	0	0	15.5	84.5
Kansas No. 6094:								
Abscission	0	117	60	1	0	65.7	33.7	0.6
Semiabscission	0	2	184	85	0	.7	67.9	31.4
Fracture	0	3	102	329	0	.7	23.5	75.8
All strains:								
Abscission	87	723	1,472	89	3.7	30.5	62.1	3.7
Semiabscission	2	160	1,910	500	.1	6.2	74.3	19.4
Fracture	0	3	1,852	5,284	0	.1	25.9	74.0

$Q = 0.909 \pm 0.001$

The data presented in Table XII for each of the nine strains show that the correlation existing between spikelet disarticulation by abscission and abundant midlength basal hairs varies in the different strains. In Kansas Nos. 5020 and 6094, 53.1 and 65.7 per cent, respectively, of the kernels having this disjunction method also had abundant midlength basal hairs. In the other four strains in which kernels having this disjunction method were observed there also was correlation between these two characters but it was not so high.

Kernels in which spikelet disjunction was by semiabscission generally were found to have few basal hairs. The correlation between these two characters varied slightly in different strains but was very pronounced in every strain grown. In only one strain, Kansas No. 6076, did less than 50 per cent of the kernels disarticulating by semiabscission have few basal hairs. These correlations might be taken as clearly indicating the heterozygous condition of the kernels in either of these classes.

The data for the different strains show conclusively that there is correlation between spikelet disarticulation by fracture and the absence of basal hairs. In seven of the nine strains more than 70 per cent of the kernels disarticulating by fracture had no basal hairs. In some strains these two conditions were associated in more than 80 per cent of the kernels.

Only 3.7 per cent of the kernels disarticulating by abscission were without basal hairs. Less than 0.1 per cent

of the kernels disarticulating by fracture had abundant midlength basal hairs, while 74 per cent of them were without basal hairs. As might be expected, it is very unusual for kernels of Burt oat to have abundant midlength basal hairs in the absence of the basal cavity.

A very high degree of association is evident between spikelet disarticulation by abscission (presence of basal cavity) and presence of basal hairs ($Q=0.909\pm0.001$). Abundant basal hairs occur most frequently on kernels having the prominent basal cavity which is characteristic of varieties of *Avena byzantina*.

SPIKELET DISARTICULATION AND AWNS

The summarized data on the correlation between spikelet disarticulation and awns for all strains are presented in Table XIII. As in the cases previously considered, in determining the value of Q, the semiabscission and abscission spikelet disjunction classes were combined. The twisted and the nontwisted long and short classes of awns also were all combined in the class "awns present," which was contrasted with the "awns absent" class, according to the following scheme:

Spikelet disarticulation by—	Awns	
	Present	Absent
Abscission and Semiabscission	4,570	373
Fracture	2,371	4,768

$Q=0.922\pm0.0009$

It is evident that high correlation exists between spikelet disarticulation by abscission and semiabscission (presence of a basal cavity) and the presence of awns. It is evident also that correlation exists between the absence of awns and spikelet disarticulation by fracture (absence of a basal cavity). These conditions are associated in about 67 per cent of the cases. There was a much higher percentage of fracturing kernels which bore long awns than of those disarticulating by abscission which lacked awns.

TABLE XIII.—*Data on association of spikelet disarticulation and awns in 9 strains of Burt oat grown at the Kansas Agricultural Experiment Station in 1920*

Strain number and kind of spikelet disarticulation in kernels	Number of kernels with awns—				Percentage of kernels with awns—			
	Twisted	Nontwisted		Absent	Twisted	Nontwisted		Absent
		Long	Short			Long	Short	
Kansas No. 5020:								
Abscission	0	127	81	1	0	60.8	38.7	0.5
Semiabscission	13	193	127	63	3.3	48.7	32.1	15.9
Fracture	55	259	120	604	5.3	24.9	11.6	58.2
Kansas No. 5211:								
Abscission	1	543	246	9	.1	68.0	30.8	1.1
Semiabscission	35	164	159	74	8.1	38.0	36.8	17.1
Fracture	187	218	77	346	22.6	26.3	9.3	41.8
Kansas No. 5219:								
Abscission	0	509	221	7	0	69.1	30.0	.9
Semiabscission	23	457	249	39	3.0	59.5	32.4	5.1
Fracture	71	73	29	226	17.8	18.3	7.3	56.6
Kansas No. 5220:								
Abscission	5	48	46	2	5.0	47.5	45.5	2.0
Semiabscission	31	61	34	43	18.3	36.1	20.1	25.5
Fracture	62	109	101	337	10.2	17.9	16.6	55.3
Kansas No. 6004:								
Semiabscission	1	13	2	2	5.6	72.2	11.1	11.1
Fracture	66	58	10	785	7.2	6.3	1.1	85.4
Kansas No. 6052:								
Semiabscission	7	9	3	2	33.3	42.9	14.3	9.5
Fracture	181	214	70	935	12.9	15.3	5.0	66.8
Kansas No. 6076:								
Semiabscission	19	29	16	26	21.1	32.2	17.8	28.9
Fracture	7	55	20	823	.8	6.1	2.2	90.9
Kansas No. 6090:								
Abscission	0	209	129	9	0	60.2	37.2	2.6
Semiabscission	0	173	173	61	0	42.5	42.5	15.0
Fracture	24	44	33	506	4.0	7.2	5.4	83.4
Kansas No. 6094:								
Abscission	0	124	54	0	0	69.7	30.3	0
Semiabscission	131	75	30	35	48.3	27.7	11.1	12.9
Fracture	173	35	20	206	39.8	8.1	4.6	47.5
All strains:								
Abscission	6	1,560	777	28	.2	65.8	32.8	1.2
Semiabscission	260	1,174	793	345	10.1	45.7	30.8	13.4
Fracture	826	1,065	480	4,768	11.6	14.9	6.7	66.8

$Q=0.922\pm0.009$

From the data presented in Table XIII it does not appear that spikelet disarticulation by semiabscission is strongly correlated with any one kind of awn, although more often kernels disarticulating by semiabscission have nontwisted long or short awns than twisted awns or awns absent. In every strain there is a marked tendency for the nontwisted long awns and disarticulation by abscission to be associated, while a comparatively high percentage of the kernels disarticulating by fracture had awns absent. The twisted awn does not appear to be associated with any spikelet disarticulation method, although there seems to be some evidence of lack of association between the twisted awn and abscission. This, together with the data on inheritance of awns in other portions of this paper, indicates that the twisted awn is possibly genetically distinct from the other awn types in the strains of Burt oat used in these experiments.

TABLE XIV.—*Data on association of lemma color and spikelet disarticulation in 9 strains of Burt oat grown at the Kansas Agricultural Experiment Station in 1920*

Strain number and lemma color in kernels	Spikelet disarticulation					
	Number of kernels disarticulating by—			Percentage of kernels disarticulating by—		
	Abscission	Semiabscission	Fracture	Abscission	Semiabscission	Fracture
Kansas No. 5020:						
Black	0	24	3	0	88.9	11.1
Brown	162	327	505	16.3	32.9	50.8
Red	38	45	461	7.0	8.3	84.7
Yellow	9	0	63	12.5	0	87.5
White	0	0	6	0	0	100.0
Kansas No. 5211:						
Black	17	4	29	34.0	8.0	58.0
Brown	418	178	358	43.8	18.7	37.5
Red	362	237	315	39.6	25.9	34.5
Yellow	2	13	126	1.4	9.2	89.4
Kansas No. 5219:						
Black	4	32	4	10.0	80.0	10.0
Brown	444	552	254	35.5	44.2	20.3
Red	239	171	141	43.4	31.0	25.6
Yellow	50	13	0	79.4	20.6	0
Kansas No. 5220:						
Black	0	7	6	0	53.8	46.2
Brown	18	101	200	5.6	31.7	62.7
Red	83	48	280	20.2	11.7	68.1
Yellow	0	13	118	0	9.9	90.1
White	0	0	5	0	0	100.0
Kansas No. 6004:						
Brown	0	17	231	0	6.9	93.1
Red	0	1	582	0	.2	99.8
Yellow	0	0	106	0	0	100.0
Kansas No. 6052:						
Brown	0	19	369	0	4.9	95.1
Red	0	2	603	0	0.3	99.7
Yellow	0	0	412	0	0	100.0
White	0	0	16	0	0	100.0
Kansas No. 6076:						
Black	0	0	11	0	0	100.0
Brown	0	52	141	0	26.9	73.1
Red	0	27	286	0	8.6	91.4
Yellow	0	11	467	0	2.3	97.7
Kansas No. 6090:						
Black	16	13	6	45.7	37.1	17.2
Brown	299	355	533	25.2	29.9	44.9
Red	32	39	57	25.0	30.5	44.5
Yellow	0	0	11	0	0	100.0
Kansas No. 6094:						
Black	2	0	0	100.0	0	0
Brown	58	139	142	17.1	41.0	41.9
Red	89	92	144	27.4	28.3	44.3
Yellow	29	40	143	13.7	18.9	67.4
White	0	0	5	0	0	100.0
All strains:						
Black	39	80	59	21.0	47.3	31.7
Brown	1,399	1,740	2,733	23.8	29.6	46.6
Red	843	662	2,869	19.3	15.1	65.6
Yellow	90	90	1,446	5.5	5.5	89.0
White	0	0	32	0	0	100.0

$Q = 0.501 \pm 0.005$.

LEMMA COLOR AND SPIKELET DISARTICULATION

The summarized data on correlation between spikelet disarticulation and lemma color, as presented in Table XIV, indicate that some correlation exists between these characters. The coefficient of association was determined in this case by combining the black and brown color classes as dark and the red, yellow, and white as light. Two disarticulation classes were combined as before, that is, abscission and semiabscission were added together in contrast to the fracture class. The data thus arranged appear as follows:

Lemma color	Disarticulation	
	Abscission and semiabscission	Fracture
Dark	3,258	2,792
Light	1,685	4,347

$Q=0.501 \pm 0.005$

The data indicate that most of the kernels, without respect to color, disarticulated by fracture. As a rule in those strains in which a high percentage of kernels of one color disarticulated by abscission, high percentages of the kernels of all colors disarticulated in the same manner.

There is association between lemma color and spikelet disarticulation or basal form. Dark kernels most often have the prominent basal cavity resulting from disarticulation by abscission, while yellow or white kernels seldom are found in strains disarticulating in this way.

The data indicate that generally the yellow and white kernels disarticulate by fracture. This agrees with the observations of Love and Craig (*71*) and others. The darker-colored kernels are found associated more often with the spikelet disarticulation by abscission while light-colored kernels are more often associated with spikelet disarticulation by fracture. The association between dark color and abscission apparently is not as strong as that between light color and fracture.

LEMMA COLOR AND AWNS

The data on the correlation between lemma color and awns are presented in Table XV. As in the cases previously described, the classes for lemma color and awns were grouped so as to make only two classes of each, as follows:

Lemma color	Awns	
	Present	Absent
Dark	4,104	1,946
Light	2,837	3,195

$Q=0.407 \pm 0.005$

The results obtained in this study indicate that the twisted awn must be different in genetic constitution from the other classes of awns. In the study of correlation between awns and lemma color it is found that practically the same percentages of twisted awns appeared in each color class with the exception of white.

If the data for the long and short nontwisted awns are combined as nontwisted, the following figures are obtained:

Lemma color	Character of awn		
	Twisted	Nontwisted	Absent
	Per cent	*Per cent*	*Per cent*
Black	7.9	64.6	27.5
Brown	9.4	58.3	32.3
Red	8.0	44.8	47.2
Yellow	10.8	21.4	67.8
White	0	6.2	93.8

The figures indicate a consistent and significant decrease in the nontwisted awn class in passing from the darker to the lighter colors.

The data for each of the strains are in general agreement with the conclusions drawn from the summary data, although in some strains the relation between lemma color and awns is less definite than in others.

The presence or absence of awns may be rather uniformly either low or high for all colors in a given strain. The variations or irregularities which occur in these strains probably may be accounted for by the fact that only a few individuals were studied.

In general, it may be stated that some correlation exists between color of lemma and the type of awn in the Burt oat used in these experiments. The lighter-colored kernels as a rule have fewer nontwisted awns than the darker kernels.

The three awn classes—twisted, nontwisted, and absent—are clearly different in breeding behavior as indicated in the studies of independent inheritance and as shown by their associations with other spikelet characters.

TABLE XV.—*Data on association of lemma color and awn class in 9 strains of Burt oat grown at the Kansas Agricultural Experiment Station in 1920*

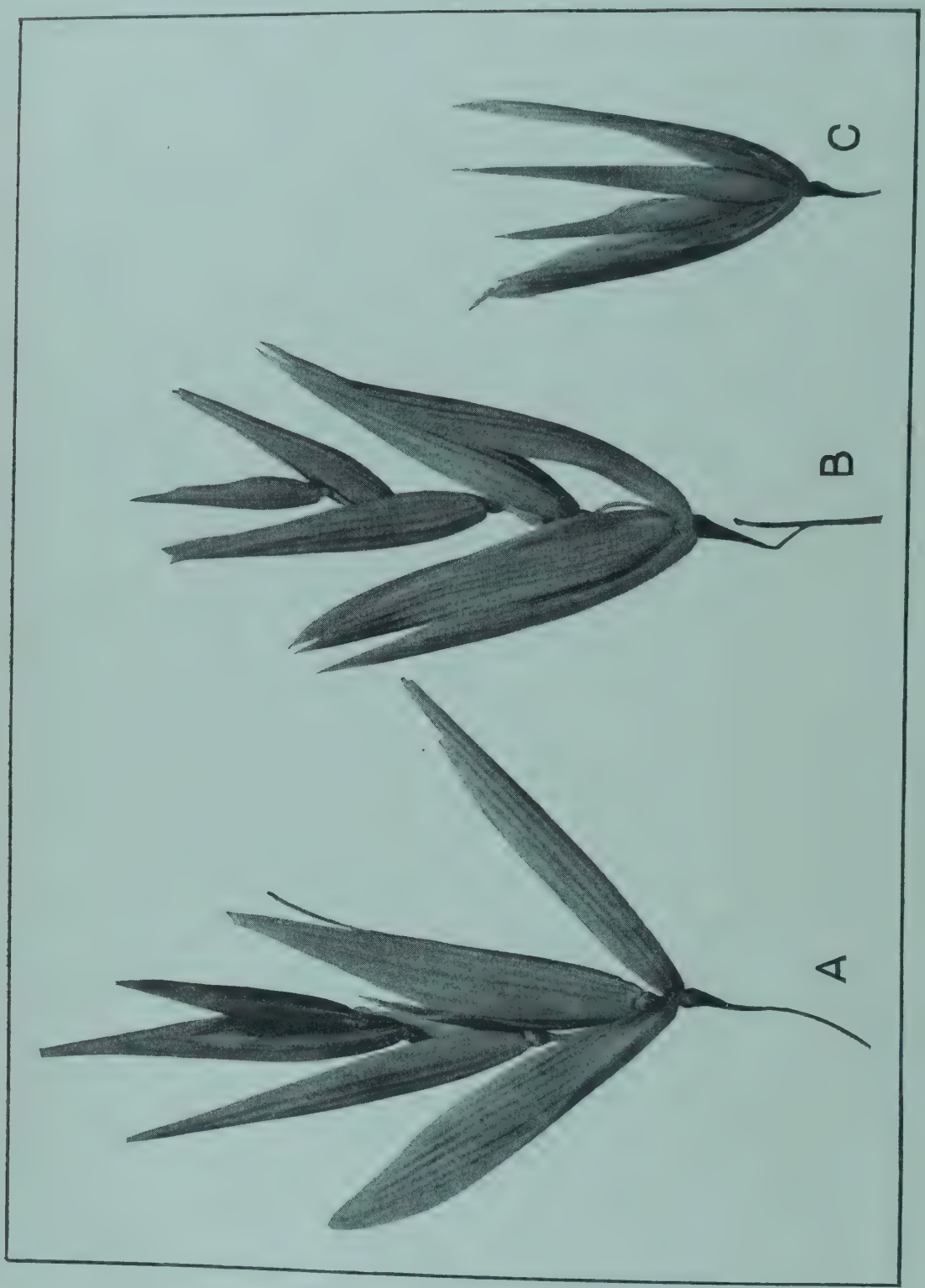
Strain number and lemma color in kernels	Awn classes in progeny							
	Number of kernels with awns—				Percentage of kernels with awns—			
	Twisted	Nontwisted		Absent	Twisted	Nontwisted		Absent
		Long	Short			Long	Short	
Kansas No. 5020:								
Black	0	7	5	15	0	25.9	18.5	55.6
Brown	63	396	228	307	6.4	39.8	22.9	30.9
Red	5	155	85	299	.9	28.5	15.6	55.0
Yellow	0	20	9	43	0	27.8	12.5	59.7
White	0	1	1	4	0	16.7	16.7	66.6
Kansas No. 5211:								
Black	11	26	9	4	22.0	52.0	18.0	8.0
Brown	138	466	180	170	14.5	48.8	18.9	17.8
Red	58	361	280	215	6.4	39.5	30.6	23.5
Yellow	16	72	13	40	11.3	51.1	9.2	28.4
Kansas No. 5219:								
Black	0	20	13	7	0	50.0	32.5	17.5
Brown	83	656	322	189	6.6	52.5	25.8	15.1
Red	11	316	148	76	2.0	57.3	26.9	13.8
Yellow	0	47	16	0	0	74.6	25.4	0
Kansas No. 5220:								
Black	3	2	2	6	23.1	15.4	15.4	46.1
Brown	62	92	53	112	19.5	28.8	16.6	35.1
Red	33	105	108	165	8.0	25.5	26.3	40.2
Yellow	0	19	18	94	0	14.5	13.7	71.8
White	0	0	0	5	0	0	0	100.0
Kansas No. 6004:								
Brown	55	51	12	130	22.2	20.6	4.8	52.4
Red	11	15	0	557	1.9	2.6	0	95.5
Yellow	1	5	0	100	1.0	4.7	0	94.3
Kansas No. 6052:								
Brown	44	74	13	257	11.3	19.1	3.4	66.2
Red	71	111	46	377	11.7	18.4	7.6	62.3
Yellow	73	38	14	287	17.7	9.2	3.4	69.7
White	0	0	0	16	0	0	0	100.0
Kansas No. 6076:								
Black	0	5	4	2	0	45.4	36.4	18.2
Brown	0	56	21	116	0	29.0	10.9	60.1
Red	25	13	7	268	8.0	4.2	2.2	85.6
Yellow	1	10	4	463	.2	2.1	.8	96.9
Kansas No. 6090:								
Black	0	16	4	15	0	45.7	11.4	42.9
Brown	8	367	287	525	.7	30.9	24.2	44.2
Red	12	43	44	29	9.4	33.6	34.4	22.6
Yellow	4	0	0	7	36.4	0	0	63.6
Kansas No. 6094:								
Black	0	2	0	0	0	100.0	0	0
Brown	98	93	57	91	28.9	27.4	16.8	26.9
Red	126	92	32	75	38.8	28.3	9.8	23.1
Yellow	80	47	15	70	37.7	22.2	7.1	33.0
White	0	0	0	5	0	0	0	100.0
All strains:								
Black	14	78	37	49	7.9	43.8	20.8	27.5
Brown	551	2,251	1,173	1,897	9.4	38.3	20.0	32.3
Red	352	1,211	750	2,061	8.0	27.7	17.1	47.2
Yellow	175	258	89	1,104	10.7	15.9	5.5	67.9
White	0	1	1	30	0	3.1	3.1	93.8

$Q = 0.407 \pm 0.005$

There is some degree of association between lemma color and nontwisted awns ($Q = 0.407 \pm 0.005$). Little if any association is apparent between the twisted awn and lemma color. This furnishes additional evidence of the fact that this awn probably belongs to a genotype very distinct from the other awn types. The light-colored kernels are more frequently awnless than are the dark-colored kernels. Nontwisted long awns are found much more numerous in the dark-colored kernel classes.

PLANT VARIANTS

These experiments have been chiefly concerned with the study of kernel characters. However, several interesting variations in plant characters have been observed. Different pedigreed lines vary considerably in the growth habit of the young plants. Some are as prostrate as plants of Red Rustproof or Winter Turf, others are semierect, somewhat resembling Fulghum, while the majority of plants have the erect



(For explanatory legend see p. 57)

habit characteristic of such varieties as Kherson and Swedish Select. Variations in size of culm, leaf width, leaf color, and other plant characters also have been observed, as well as distinct differences in time of heading and of ripening. A few plants were observed among the material grown at Akron Field Station in 1921, which appeared to have the growth habit of true winter oats and which failed to produce heading culms.

A few of the more unusual plant variations will be described in a little greater detail because of their relation to the general problem of variability in Burt oat. It is expected that the breeding behavior of some of these variants will be more fully described in other papers. It is interesting to note that while more plant variations have been observed in Burt than in almost any other variety, no dwarf forms such as have been described by Warburton (*145*) and Stanton (*125*) have yet been observed in Burt. It also is noteworthy that only a few false wild types have been observed in these experiments on variability in Burt oat, although they frequently occur in some other varieties of *Avena byzantina*.

CHLOROPHYLL DEFICIENCY

The first of these variations observed exhibited a chlorotic condition, manifested in the form of leaf striping. Chlorophyll abnormalities in small grains have been described by Nilsson-Ehle (*94, 97*), Kalt (*56*), Kiessling (*59, 60*), Akerman (*3*), Christie (*19*), Wiebe,[22] and others. East and Hayes (*31*), Emerson (*32*), Gernert (*46*), Miles (*78*), and Lindstrom (*65, 66*) have described various chlorotic types in corn.

The original showing this striped condition of the leaves was first noticed in 1920 at the Akron Field Station shortly after the plant emerged, and throughout the life of the plant, as far as could be observed, the proportionate area of the leaves affected remained the same, neither increasing nor decreasing as the plant developed. This plant made only about one-half the growth attained by the normal green plants from the same parental strain and matured a single panicle bearing a few kernels.

Seeds of this plant were sown in 1921 at the Akron station, but out of 13 seedlings only 1 plant reached maturity. Some of these plants appeared to be white, or entirely devoid of chlorophyll. The 1 plant grown to maturity in 1921 showed the striped condition similar to the original variant, but the striping was less marked. The seeds of this plant were sown in individual pots in the greenhouse of the Kansas Agricultural Experiment Station at Manhattan in the fall of 1921. The chlorotic condition was exhibited in varying degrees by a considerable number of the plants grown. No albinos appeared among these progeny plants.

Seed from each of the plants grown to maturity in the greenhouse at Manhattan in the winter of 1921–22 was sown at the Akron station in 1922. About 800 plants were grown, of which a considerable percentage exhibited the chlorotic condition in varying degrees, ranging from only a few rather indistinct longitudinal yellowish-white stripes to the apparent total absence of chlorophyll.

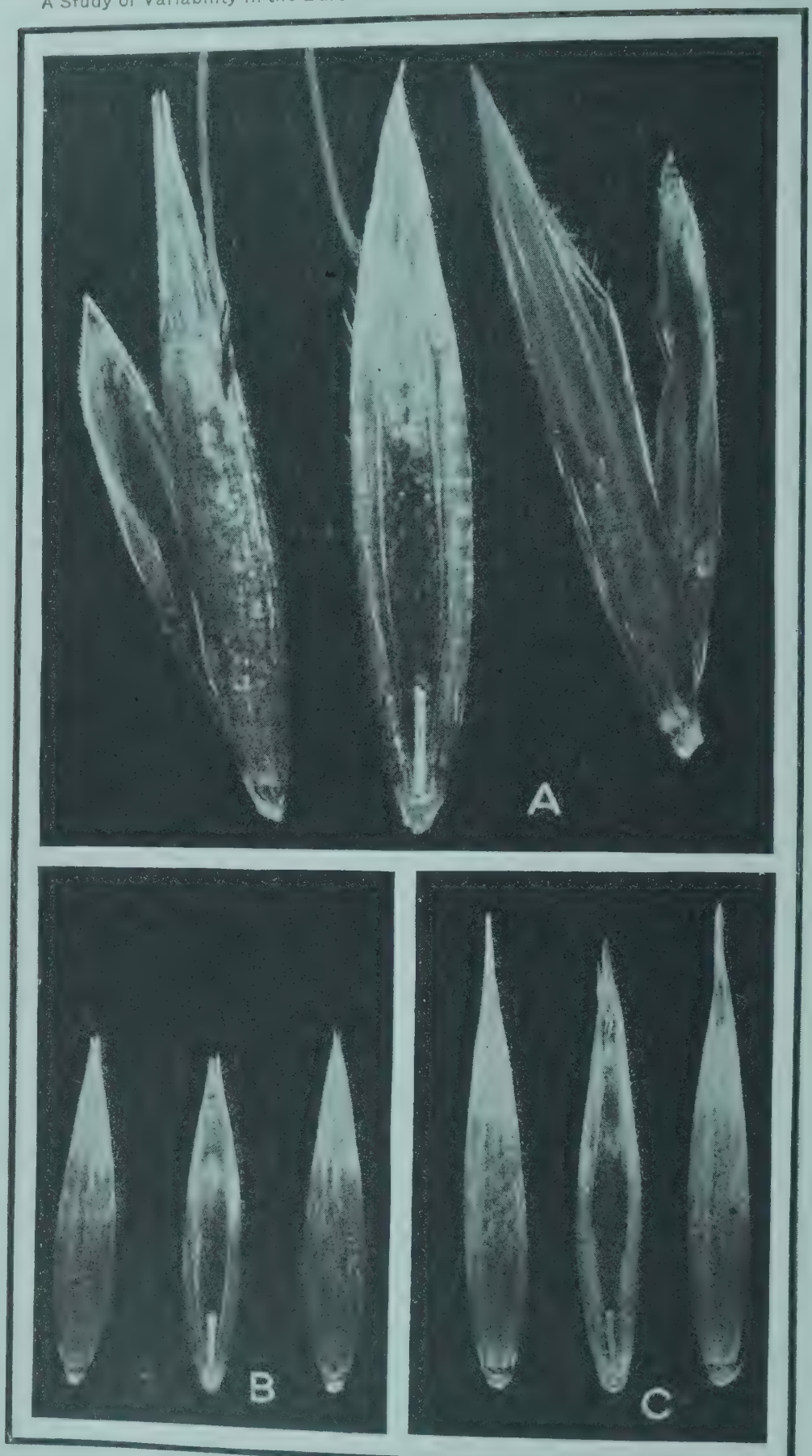
MULTIFLOROUS SPIKELETS

A second variant type was observed in 1920 at Akron. It had the multiflorous spikelets which Stanton (*124*) has shown to characterize the hull-less or naked oat. This variation has been described by Coffman and Quisenberry (*20*). The outer glumes were long, resembling those of *Avena nuda*. The spikelets were multiflorous, containing from two to six florets per spikelet which Spillman (*122*) has reported that Von Tschermak found to be correlated with the hull-less condition of *A. nuda*. Approximately 40 to 50 per cent of the kernels threshed free from the glumes as in *Avena nuda*. The plant was similar in general appearance to the F_1 plant of a cross between a hulled and a hull-less oat, as described by Norton (*99*), Zinn and Surface (*161*), Gaines (*40*), Caporn (*14*), and Love and McRostie (*73*). All of these writers have observed that the F_1 plants of the hulled × hull-less cross exhibit an intermediate condition and that hulled and hull-less kernels are found in the F_1 plants in varying percentages. Caporn (*14*) has observed a definite arrangement of hulled and hull-less kernels in the various parts of the panicles of the F_1 plants. No such arrangement was observed in this variant plant nor in the progeny grown from it.

[22] WIEBE, G. A. A CASE OF ALBINISM IN BARLEY. Jour. Heredity. 15:221–222. 1924

EXPLANATORY LEGEND FOR PLATE 7

A.—Multiflorous spikelet of Burt oat, Kansas No. 6090, resembling *Avena nuda*. Note the dark color and firm texture of lemmas of upper florets. B.—Typical multiflorous spikelet of hull-less oat, *Avena nuda*. C.—Typical spikelet of Burt oat, Kansas No. 6090.



A.—Loose palea from Kansas No. 6076 approaching the condition found in the hull-less oat. B.—Short and very broad kernels from Kansas No. 6052. C.—Long and broad kernels from Kansas No. 5220.

Plants of this variant type were grown at the Akron and Manhattan stations in 1921 and at the Akron Field Station and the West Virginia Agricultural Experiment Station in 1922. These experiments have shown that this multiflorous condition is heritable, though there is considerable variation in the progenies with respect to the percentage of kernels remaining inclosed in the lemmas after threshing. A spikelet of this variation is shown in Plate 7, in comparison with those of normal Burt oat and *Avena nuda*, the hull-less oat.

LOOSE PALEAS

Several plants were observed among the 1920 progeny at both the Akron and Manhattan stations in which the paleas of the kernels did not closely inclose the caryopsis. In some of these the palea was curled away from the lemma, leaving the caryopsis exposed. Such kernels were seeded in 1921 at both stations and the plants grown proved the condition to be heritable to some extent. Plate 8 shows several of these kernels.

FALSE WILD OAT

Criddle (*24*) observed false wild forms in the Early Ripe oat, a variety very similar to Burt. These false wild forms have been discussed by Fischer (*35*), Norton (*98*), Criddle (*24, 25*), Nilsson-Ehle (*93, 96*), Newman (*86, 87*), Zade (*153, 154*), Atwood (*6*), Von Tschermak (*137*), Pridham (*106*), Robb (*113*), Agar (*1*), Gante (*41*), Hayes and Garber (*47*), Crepin (*23*), Akerman (*2*), Garber (*42*), and Garber and Quisenberry (*43*). A few plants bearing kernels resembling the false wild forms described in publications named above were first observed in the material grown at Akron in 1920. More recently a number of such aberrant individuals were found in Burt at Akron, and one was selected from a strain of this variety by W. H. von Trebra at the Colby (Kansas) Substation in 1924. In these forms the basal cavity was very prominent on the upper as well as the lower kernels of the spikelets. Both the primary and secondary kernels bore awns which were markedly twisted. Abundant hairs practically surrounded the base of the lemma and were present on the rachillas of both kernels. The presence of a few hairs also was observed on the dorsal surface of some kernels. The false wild kernels were of a yellowish-red color. Kernels of false wild oats which occurred in selection No. 16–3 of Kansas strain No. 6076 of Burt grown at Akron in 1920 are illustrated in Plate 9.

SUMMARY

A review of the literature on classification and breeding experiments with oats is presented, with special reference to the characters considered in this paper.

Experiments of different investigators have shown that certain strains of Burt oat are resistant to crown rust and to smut. These characters add to the value of the variety.

The Burt oat has considerable economic value due to its wide adaptability, early maturity, drought resistance, and resistance to smut and crown rust.

The commercial Burt oat is composed of a large number of distinct strains and many of these are heterozygous. This variety has been classified as *Avena sativa* by Carleton (*17*), *Avena sterilis* by Etheridge (*33*), and in other ways by other investigators. There seems to be little basis for the suggestion of Waller (*141*) that the Burt oat belongs to *Avena barbata*.

Burt may belong to a distinct group, as suggested by Norton (*98*), but the writers consider it as belonging to *Avena byzantina*, the species accepted by many European taxonomists as including cultivated descendants of *Avena sterilis*. This species is recognized as being variable in its breeding behavior and as containing strains which resemble those of *Avena sativa*.

Investigators do not agree concerning the amount of natural crossing which takes place in oats. The writers believe that field crosses may occur rather frequently, the amount of natural crossing varying with the variety and environmental conditions.

Spikelet disarticulation in the Burt oat appears to breed as a simple monohybrid. The roughened type of base resulting from fracture, very similar to that of *Avena sativa*, apparently is dominant. The base resulting from abscission and containing a prominent cavity apparently is recessive.

Two types of floret disjunction are found in Burt oat, the *sativa* or disarticulating form predominating. The *byzantina* or basifracture form, in its breeding behavior, somewhat suggests a cross involving multiple factors.

The development of basal hairs in Burt is a character which appears to be complex in breeding behavior. Probably several factors are concerned. The abundant long hairs appear to be recessive.

Several factors probably determine the breeding behavior of awns in the Burt oat. The twisted awn bred more nearly true than did any of the others.

This type of awn behaves as a recessive character and apparently is distinct genetically from the other types of awn.

The results obtained on the inheritance of lemma color in the Burt oat indicate that red is the most stable in breeding behavior. The dark browns and blacks tend to produce a high percentage of dark kernels and seem to have a similar genetic constitution. Some of the kernels of the 1920 crop described as light brown and yellow probably were genetic reds manifesting the effects of physiological influences. Other workers have shown that white is a recessive kernel color in oats. In the experiments of the writers, however, the parental kernels classed as white did not produce a high percentage of white progeny kernels. This may have been due to weathering, immaturity, and physiological influences which prevented the development of, or which obscured, the true color.

In the study of association between spikelet characters in Burt oat it has been found that correlation exists among the following characters: (1) Floret disjunction and spikelet disarticulation; (2) spikelet disarticulation and basal hairs; (3) spikelet disarticulation and awns; (4) lemma color and spikelet disarticulation; (5) lemma color and awns.

Burt oat has been shown to vary in many observable plant characters, such as habit of growth of the young plant, leaf color and leaf width, time of heading and ripening, and others. Several distinct variants have been observed, among them one showing a chlorotic condition of the leaves, one having multiflorous spikelets, one with loose paleas, and the false wild forms.

These experiments provide a basis for future breeding experiments with Burt oat. The possibility of isolating comparatively pure-breeding strains from the variety is pointed out. The use of strains of Burt oats as parental material in genetic experiments without first carefully observing their breeding behavior for several years in pedigree culture is shown to be not in accord with accepted genetic methods.

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EXPLANATORY LEGEND FOR PLATE 9

A.—Dorsal and ventral views of kernels of the so-called "false wild" oat showing long and twisted geniculate awns. B.—Enlargement of basal portion of one kernel of the so-called "false wild" showing characters common to wild oat species, namely, disarticulation by abscission, (1) with resulting basal cavity with smooth margins; (2) floret disjunction by disarticulation, leaving the second rachilla segment attached ventrally to the lower floret, and (3) abundant long hairs on the rachilla segment

(For explanatory legend see p. 60)

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THE COMPOSITE LIFE HISTORY OF PUCCINIA PODOPHYLLI SCHW.[1]

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The common rust, *Puccinia podophylli* Schw., presents certain features which make it of special interest to the student of the biology of this great group of plant parasites. The interest in this species lies primarily in the peculiar sequence in the seasonal appearance of the spore forms. This species is an opsis-form, possessing aecia and telia only, and occurs on the common mandrake or May-apple, *Podophyllum peltatum* L., practically throughout its range.

Puccinia podophylli produces teliospores at two different periods during the season; the first crop appears early in the spring on the sheath leaves or bud scales, on the stem usually at the base and sometimes on the sepals (Pl. 1, A and B); the second crop appears later in the summer on the under side of the fully expanded and matured leaves (Pl. 4, B). Between these two crops of teliospores the aecia, usually preceded by pycnia, are developed on the expanding leaves (Pl. 2). It is also of interest that telia often develop in association with the aecial clusters on the leaves and to all appearances on the same mycelium before the appearance of the second crop of teliospores, which obviously develop from aecial infection (Pls. 2, 3, and 4, A). The explanation of the origin of these early telial sori and the determination of the life history of this species are the objects of the study reported in this paper.

HISTORY

The only previous attempt to explain the life history of this species and the sequence of the spore forms was made by Olive (*6*). Based primarily on cytological evidence, he reached the conclusion that the first crop of telia and the pycnia and aecia arise from an intermingled growth of the mycelium of the gametophytic and sporophytic generations which are independently and simultaneously perennial in the host plant. He conducted no culture work nor did he attempt to verify this assumption by an histological study of the root stalk or overwintering buds.

The following summary of Olive's work is presented in some detail, largely by quotation, in order to bring out clearly his cytological observations and the conclusions he drew from them since it will be necessary to refer to these details later in this paper.

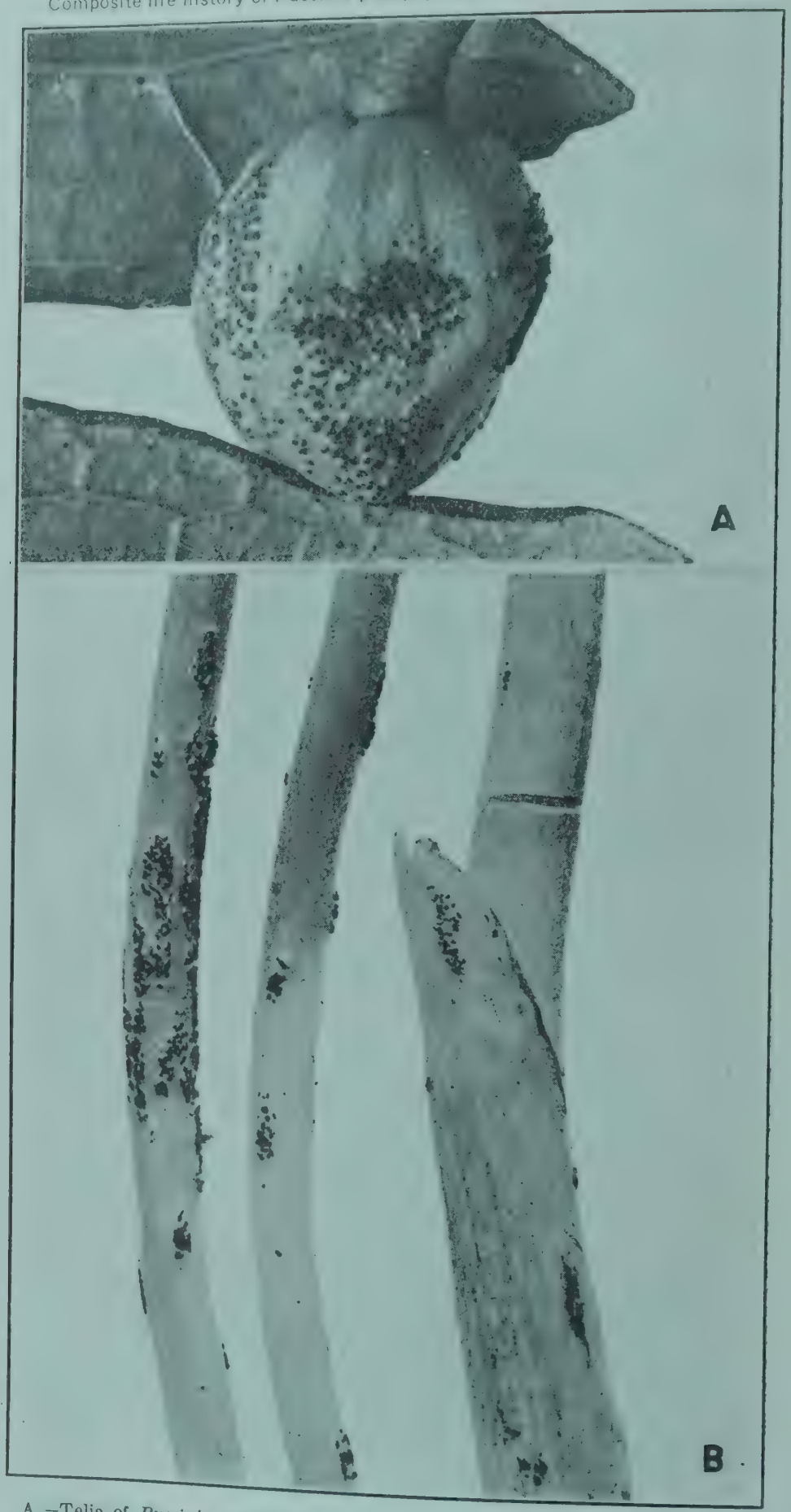
Olive found that the mycelium in the lesions on the sheath leaves that bore the early telia was prevailingly binucleate, as one would expect.

> The preparations also show, occasionally, a few aecidium cups on these same sheaths * * *. I have not yet found spermogonia on the sections of these young sheaths; but their occasional occurrence in such situations may be expected from the fact that a small amount of uninucleate mycelium occurs, especially in the region surrounding the aecidial sori, there forming the meager pseudo-parenchyma. But the rust mycelium of the sheath, in contradistinction to that in the young leaves, is undoubtedly prevailingly binucleate. Further, I

[1] Received for publication May 24, 1924—issued April, 1925. Joint contribution from the Department of Botany, Purdue University Agricultural Experiment Station and the Department of Plant Pathology, Cornell University.

[2] A report on the experiment conducted at Ithaca, N. Y., together with an interpretation of the life history essentially the same as that given in this paper, was presented before the Botanical Society of Ameriea at the Pittsburgh meeting of the American Association for the Advancement of Science in 1917 by Prof. H. H. Whetzel (not published). The problem had been freely discussed with me during the two previous years and my interest aroused. It was mutually agreed that a more detailed cultural study would be desirable and this work was begun at LaFayette, Ind., in 1917, having been planned in collaboration with my associate, Dr. E. B. Mains, who carried out the details of the cultures. It was finally decided to present the combined results in a joint paper, the preparation of which has been my responsibility.—H. S. Jackson.

[3] Reference is made by number (italic) to "Literature cited."



A.—Telia of *Puccinia podophylli* on the sepals. Note the acciallike character of these telia. Natural infection slightly enlarged
B.—Telia on bud scales and on the stem. Natural infection. Compare with Plate 4, B. Natural size.

am convinced that the aecidia which are borne on the sheaths arise, not from gametophytic cell fusions, but only from preëxisting binucleate hyphae; therefore being secondary and sporophytic in character, and thus similar in origin to the teleutospores.

He found in the lesions on the leaves that a much more abundant uninucleate mycelium is present in the younger tissues and that the pycnia arise from this mycelium.

In those older leaves, however, in which the aecidia have begun to form their chains of spores, binucleate mycelium has become quite prevalent in all the sections examined, especially at the bases of the aecidial cups. These sporophytic hyphae intermingle with the uninucleate mycelium, often entering the broad, caeoma like base of the young aecidium, there functioning directly as basal cells of the rows of the binucleate aecidiospores. In still older stages on leaves, binucleate mycelium apparently prevails by the time the aecidium cups have for the most part broken open to discharge their spores, * * *.

His examination of the later telia which form from aeciospore infection shows that they arise from a localized binucleate mycelium.

In interpreting the results of his observations he assumes that the uninucleate, or gametophytic mycelium, and the binucleate or sporophytic mycelium, are independently but simultaneously perennial in the plants from which his material was taken and that the pycnia are the only structure borne on the uninucleate mycelium, while both the early teliospores and the aecia develop from the binucleate mycelium. He assumes that basidiospore infection would result in a localized mycelium on which pycnia and aecia would be developed, the expectation being that this would be prevailingly uninucleate.

In connection with a discussion of the principle that in perennial rusts the gametophytic mycelium in young shoots grows more vigorously early in the season than the sporophytic, the following quotation is of interest:

The fact that the teleutosori break out very early from the tissues of the leaf sheaths, some distance below the tip of the stem, and that the binucleate mycelium prevails in these sheaths almost to the exclusion of the uninucleate hyphae does not, to my mind, vitiate the above statement, which applies only to the younger tissues of the shoot. I interpret these facts somewhat as follows: the uninucleate mycelium grows with especial vigor into the rapidly expanding tip and young leaves of the new shoot, growing somewhat ahead of the lagging sporophyte. The latter apparently chooses ordinarily the more mature tissues for its most vigorous growth and thus early comes to predominate in the riper tissues of the poorly nourished leaf sheaths of Podophyllum, as well as later in the older leaf tissues.

As a part of Olive's discussion of the probable reason for the development of the early telia on the sheaths and later the secondary aecia on the leaves from the same binucleate mycelium, the following significant statements are found:

Quite likely the degree of maturity of the tissues and the quality of the nourishment supplied to the parasite govern largely this phenomenon of the early development of the teleutospores, just as these factors doubtless determine the cessation of the production of the repeating spores and the beginning of the production of the teleutospores in other long-cycled rusts. The tissues making up the leaf sheaths of young shoots of Podophyllum are undoubtedly mature, as well perhaps as poorly nourished, as is shown by the character of the contained protoplasm; hence they present conditions at a very early stage not met with again until the host gains maturity or even old age.

It is obviously quite impossible to suppose that this early teleutosporic stage in *Puccinia podophylli* has arisen in any other way than from hibernating sporophytic mycelium, which has grown up with the infected buds in the spring. It could not come from an early infection since the aecidiospores, which we assume to be the only spores from the inoculation of which the teleutospores could arise, have not yet begun to form. I will have to confess, however, that I have not been able to apply de Bary's test to this species and to look for hibernating mycelium in the underground parts.

CULTURE EXPERIMENTS

EXPERIMENTS AT ITHACA, N. Y., 1916–1917

One of the authors of this paper (Whetzel) has had Podophyllum rust under observation since about 1906. During 1912 he collected and fixed much of the material on which Olive's cytological work was based and furnished him without reservation all the information gained from the field observations which had then been made. This assistance is freely acknowledged by Olive in the paper referred to above.[4]

The elaborate, though entirely orthodox, explanation of the situation finally made by Olive did not seem to Whetzel adequately to explain the phenomena which had been observed in the field. On this account it was decided to test the validity of Olive's hypotheses by culture experiments. The first of these was conducted at Ithaca, N. Y., during 1916 and 1917.

The plants used for the experiment had been brought several years before from a patch of Podophyllum in which no rust was to be found and were planted on a wooded bank in Whetzel's yard far away from any other patches of Podophyllum. Since the planting was along the path by which he reached the road on his way to the office each day, he was able to determine by careful inspection each year that no rust ever appeared either on the bud scales, stems or leaves of these plants. In the summer of 1916 he collected a large quantity

[4] A detailed manuscript record of these experiments, together with the correspondence which passed between Prof. Whetzel and Dr. Olive on the Podophyllum rust, is deposited in the library of Cornell University under the title, "Notes on the Life History of Puccinia podophylli," by H. H. Whetzel.

A.—Upper surface of leaf of *Podophyllum peltatum* inoculated with teliospores from stem sori. Note abundance of telia in aecial lesions and telia at base of stem
B.—Upper surface of aecial lesion showing telia along the veins. Natural infection
C.—Lower surface of same lesion as B, showing aecia. Natural infection

of Podophyllum leaves which bore the second, or summer crop, of teliospores in abundance, put them between two sheets of window screen and placed this over one end of the established rust-free planting in his yard. This plot had developed so that it was about 8 feet long and rather narrow. The screen packet was about 12 by 18 inches. During the fall and winter the Podophyllum leaves in the screen rotted and the spores fell with the débris through the meshes upon the surface of the soil. Early in the spring of 1917 the empty screen was removed and careful observations were made on all the plants in the plot at frequent intervals as they developed. Telia soon appeared on the sheath leaves and stems of nearly all the plants which came up in the area covered by the screen packet. No rust appeared on plants elsewhere in the bed during the entire season. A seedling in the infected area also developed aecial lesions on the leaf blade in the spring of 1917. Two nearby seedlings showed telia only on the stems. One of these seedlings stood directly under that bearing aecia and when all three were removed on June 4, 1917, it showed the whitish lesions of the developing but unopened summer telia. However, no summer crop of telia appeared on any of the other plants in the patch during 1917. In 1918, while leaf sheath and stem lesions bearing the early telia were common, only a single plant developed an aecial lesion. This was allowed to remain, and later nearly every leaf blade in the patch showed a more or less abundant development of the summer telia. Since that time this patch of plants has been rusted more or less each year, all stages of the rust appearing in the manner usual for this species.

This experiment, while not in itself conclusive, strongly suggests that the interpretations of the life history made by Olive are not in accord with the facts. It would appear from this experiment that the infection on the sheaths and stems, resulting in the early crop of telia, arose directly from the basidiospores developed from the late crop of teliospores which were used as the inoculum and that these teliospores were responsible also for infection on the seedling leaves, resulting in pycnia and aecia. Since the aecial-bearing seedling was removed along with the seedling showing the initial stages of the summer telia, and since no other plant in the patch showed summer telia during 1917, it would further appear that the spring crop of teliospores was responsible the next season for the development not only of another crop of early teliospores on sheath and stem but also for pycnia and aecia later.

EXPERIMENTS AT LA FAYETTE, IND., 1917–1921

Since this experiment was conducted entirely out of doors and the usual objections to such experiments would naturally arise, it was decided to repeat the work with variations, in part at least under controlled conditions, and to conduct this work in connection with the rust investigations under way at the Purdue University Agricultural, Experiment Station at La Fayette, Ind.

EXPERIMENT 1.—During the summer of 1917 patches of Podophyllum were located in the woods, some of which were heavily rusted and some of which showed no rust. An equal number of plants, about 25, were dug in November from each of two plots, one of which was rusted and the other not. The rhizomes were very carefully washed and cleaned of all soil or dead parts and planted in a single row in a vegetable garden (Jackson's), with a stake dividing the two lots. A frame 18 inches by 48 inches made of boards 4 inches wide was placed on the ground in the middle of the row in such a position as to include 5 plants from the rusted patch and 5 from the one which showed no rust. A peck or less of surface soil and débris taken from a patch of Podophyllum which had been heavily rusted during the previous summer was evenly spread over the soil in this frame. No protection was given the plants during the winter.

In the spring of 1918 all 10 plants in the frame showed the early telia on the bud sheaths or stems and later about half of them showed pycnia and aecia in fair abundance on the blades of the leaves. No infection of any sort occurred on the other 40 plants at either end of the frame. The experiment was not continued since the ground was needed for other purposes.

Were the rust perennial in the rhizomes some of the plants outside the frame which had been dug from the rusted patch should have been rusted. The infection on the plants in the frame presumably resulted from the teliospores present in the top soil used as a mulch.

EXPERIMENT 2.—In November, 1917, about 50 rhizomes dug from a patch of Podophyllum which showed heavy infection on nearly every plant during the spring were thoroughly washed.

Some of these were placed in flats, covered with potting soil, and placed out of doors with a heavy covering of straw. Some were potted at once and placed in the greenhouse. The material which was placed in flats was brought into the greenhouse in March, 1918, when the leaves were just emerging from the sheaths. No rust developed on either set of plants, though they were grown to maturity.

In March, 1918, before the buds had swollen to any extent, a second lot of rhizomes were dug from a patch which was known to have been heavily rusted during the previous summer. These were thoroughly washed, potted in potting soil, and allowed to develop in the greenhouse. No sign of rust infection appeared on any of them.

An inspection of the remaining plants in the patch of Podophyllum from which the last-mentioned rhizhomes had been dug was made at intervals during the spring and summer of 1918 and the plants were found to be heavily infected, showing the early development of telia on the sheaths and stems, pycnia and aecia later on the leaves, and finally the late crop of telia on the under side of the leaves.

Stems showing the early telial sori and the attached rhizomes were collected in the spring of 1918 and were studied in an effort to determine the extent of the mycelium. Free-hand sections were made, beginning in the region of the sori and progressing down the stem into the rhizome. The sections were mounted in chloral hydrate and iodine, which furnishes a satisfactory medium for the study of such sections. The mycelium is easily distinguished by this method and was found only in the immediate region of the sori. No mycelium was discovered lower down in the stem or in the rhizome.

This series of experiments taken in conjunction with the one made at Ithaca would seem effectively to dispose of the hypothesis put forward by Olive that the rust is perennial in the buds or rhizomes.

A number of other experiments were considered desirable, however, in order to determine as accurately as possible just what did occur. The question naturally arose as to whether there might be some difference in the teliospores formed early in the season on the stems and sheaths and those formed later from aeciospore infection, either in their ability to cause infection or in the sequence of resulting spore forms. It was also considered desirable to conduct some of the experiments with somewhat more refined methods and under as strict control as is feasible in dealing with growing plants and with a fungus which can not be grown in pure culture.

Experiment 3.—The telial material which was used for the following inoculation experiments was obtained in the spring and summer of 1917 in the same patches of Podophyllum from which the rhizomes used in the previous experiment were taken. The first material was collected May 28, 1917, and consisted entirely of sheaths and stems bearing the early crop of telia. These were placed out of doors on the surface of potting soil in flats and left till the following spring, 1918. On July 17, 1917, material of the late crop of telia which develop from aecial infection and occur abundantly scattered on the under surface of the leaves was collected and handled in the same way as the sheath and stem material mentioned above. Finally, in the fall of 1917, after the Podophyllum plants had died down and the leaves were more or less disintegrated. surface soil from a patch of heavily infected Podophyllum was collected and placed out of doors to winter. At the same time rhizomes were again collected from a patch of Podophyllum which had shown no infection during the year and were placed in flats, covered with potting soil, and left out of doors with protection during the winter. In March, 1918, these rhizomes were brought into the greenhouse, thoroughly washed, and potted. Some of the pots were given a surface coating of the surface soil obtained from the infected Podophyllum patch, some were mulched with the soil and stems from the flat prepared in May, 1917, and some with the soil and leaves from the flat prepared in July, 1917, as described above. It should be pointed out that the sheaths, stems, and leaves had largely disintegrated after overwintering and most of the teliospores were mixed in the surface soil of the flats in which they had been kept; hence the reason for using the soil as a mulch. As the buds of the potted plants developed they pushed up through this mulch and became exposed to infection.

The results of this experiment were as follows: In one of the pots mulched with soil, sheaths, and stems bearing the early teliospores, pycnia and a few aecia developed on the leaves of one plant and pycnia and aecia together with a few telia immediately associated with the aecia on another. In the pots mulched with soil and leaves bearing

the late crop of teliospores, four plants showed pycnia and aecia on the leaves in numerous spots, some of which on all four plants in later stages of development showed accompanying telia. In the pots mulched with soil from the infected patch one plant showed a few pycnia and abundant telia on sheath and stem, together with pycnia and aecia on the leaves in numerous spots in association with which telia were not uncommon, and the other plant showed numerous spots with pycnia and aecia, with some of which were associated telia. In all cases where telia accompanied the aecia on the leaves they were most commonly found on the veins which were involved in the areas of aecial infection. A control series of pots in which the rhizomes were mulched with ordinary potting soil showed no infection of any sort.

EXPERIMENT 4.—In this experiment overwintered teliospores from the late crop on the leaves, which by previous test were known to be germinating, were sown at three different dates on Podophyllum plants taken from an uninfected patch. This work was all done in the greenhouse. The first sowing was made on April 27, 1918, on leaves and stems of four plants, three of which had the folded leaves well out of the sheaths and the fourth just emerging. The result showed development of pycnia and aecia on leaves of all plants and telia on the stem of one. Epiphyllous telia developed in a number of the aecial areas, mostly on the veins. The second sowing was made on May 6, 1918, on six plants of Podophyllum all of which were well out of the sheath, with the leaves partly expanded. Pycnia and aecia were developed on the leaves of all plants. Some of the aecial lesions on each plant showed telia in immediate association. In one case telia were developed in association with pycnia without aecia being present. In the third sowing on May 11, made on the leaves of more mature plants, numerous lesions bearing pycnia and aecia developed on two of the plants. Telia were also developed later in association with the aecia on a majority of the spots. On both plants a few spots containing aecia developed in association with which no pycnia were observed.

EXPERIMENT 5.—This experiment dealt with the infection from aeciospores. Three sowings were made under control conditions in the greenhouse. The first sowing was made on May 28, 1917, using aeciospores collected in the field from plants which showed only aecia and with which no pycnia were observed. The inoculation was made on the expanded leaves, and fully mature telia developed on June 9. The second sowing was made on May 3, 1918, using aeciospores from plants collected in the field on which the aecia were accompanied by pycnia. Sowings were made on one mature plant and three younger plants. An attempt was made to inoculate the sheaths and stems as well as the leaves. Telia appeared, however, only on the leaf blades of the three younger plants, being fully mature on May 20. A third sowing was made on May 10, 1918, using aecia obtained from the inoculations described in Experiment 3. The inoculation was made on several plants in various stages of maturity. Telia developed only on the leaves of the younger plants.

In all of these sowings the telia obtained were of the type occurring gregariously in spots on the under side of the leaves, commonly found in the field in midsummer, and referred to in this paper as the late crop of telia.

These experiments confirm those of Arthur (*1*) and effectively dispose of the idea that repeating æcia might be present in the life history of the Podophyllum rust.

LATER EXPERIMENTS.—During 1919, 1920, and 1921, other experiments were conducted, which confirmed the results of those outlined above in all details. In a few cases telial material which was observed to be germinating in hanging-drop tests was transferred to the plant. The results were the same as where less refined methods were used.

It may be noted that only a few records of infection on the sheath leaves are made in these experiments. This, it should be explained, is due to the fact that some difficulty was encountered, under rather dry greenhouse conditions, in preventing the sheaths from drying up before the telia developed. In many such cases, however, evidence of infection was observed, and in the later experiments of 1919 to 1921, this phase of the problem was given special attention.

SUMMARY OF RESULTS OF EXPERIMENTS

A careful analysis of the results of these experiments shows that the writers have obtained the development of telia on sheaths and stems, and pycnia and aecia with associated telia on the leaves, by inoculating with germinating teliospores of both the early and the late crops.

No basis was found for the idea that the rust is in any sense perennial or that any condition approaching an "unlimited infection" is evident. It would appear that all of the structures develop from a localized mycelium and that this mycelium in all cases (except the late crop of telia) arises from basidiospore infection. It is also evident that the first spore structure borne on this mycelium may be either telia, or pycnia accompanied by aecia, and in rare cases aecia without pycnia.

These experiments when taken together with field observations made at Ithaca, N. Y., and La Fayette, Ind., would seem to make necessary an interpretation entirely different from that made by Olive.

LIFE HISTORY AND SEQUENCE OF SPORE FORMS

It seems desirable, therefore, as a basis for further discussion, that the writers here set forth in some detail what they conceive to be the regular sequence of events in the life history of this rust.

In the spring, at the time when the buds of the Podophyllum plant are emerging from the ground, the teliospores of *Puccinia podophylli* are present in the surface layer of soil around the developing buds. These teliospores are derived both from the spring crop of telia on leaf sheaths, stems, and sepals and those associated with aecial lesions, and from the summer crop produced on the leaf blades. They have either fallen to the ground during the previous summer, as the telia are of the pulverulent type, the pedicles breaking close to the spore, or they have been liberated by the rather complete disintegration of the host tissues. The spore wall is beset with sparsely scattered rather long spines which would serve to prevent the spores from being carried too deeply into the soil or washed away during heavy rains.

At the time when the sheathed leaf buds begin to push their pointed tips above the surface of the soil, these teliospores (of both crops) begin to germinate in the normal way for teliospores, that is, by the development of a typical promycelium and sporidia. The scale leaves form a sheath about the base of the stem, through which the young plant emerges. These scale leaves usually extend slightly above the ground level. The lobes of the two peltate leaves of the flowering stalk are folded about the stem in a convolute manner and the single blossom bud is partly exposed.

The basidiospores fall upon the portions of the plant which are exposed in the early stage of development and infection results. The sheath leaves or bud scales are the first organs exposed. As the aerial stalk pushes forth from within the enfolding bud scales, both the base of the stem and the leaf blade are also exposed to infection. The upper part of the stem is rarely infected as it long remains protected by the enfolding leaf blades. The two sepals of the flower are, however, not uncommonly infected, for they are usually partly exposed as the young plant emerges (Pl. 1, A and B).

The period of basidiospore production (that is, teliospore germination) apparently is relatively early and short. Very numerous infections occur on the scale leaves and on the base of the stems. This is perhaps due to the fact that they are nearer the soil surface and in the more effective zone of basidiospore dissemination. At Ithaca it has been noted in some seasons that only certain scale leaves, such, for example, as the second from the top, show infection. This observation (Whetzel's) was referred to by Olive as a "curious fact." The explanation is, however, comparatively simple. These "second" scale leaves were the only ones exposed in the particular patch under observation at the time of inoculation. There are three or more of these buds scales at the base of the stem. The top scale had not emerged from within the protecting outer second scale and the shorter third scale was still covered by leaf mold. It has since been observed, in the same patch, that all the scales and the base of the stem may be infected. It would appear that these differences are the result of seasonal variation in the coincidence of basidiospore production and development of the host and of variations in the extent of exposure of the bud scales above the surface of the leaf mold.

From the basidiospore infections on the scale leaves, the base of the stem, and the sepals, telial sori are developed almost exclusively. Pycnia and aecia are occasionally found intermixed with the telia, the latter usually occurring singly. Both are, however, rather rare, and by far the greater number of lesions include telia only. In one case noted at LaFayette an isolated lesion on the stem bore a group of pycnia surrounded by a ring of telial sori, exactly similar in appearance to many true microforms of Puccinia.

The occasional development of pycnia and aecia among the telia on sheath

leaves is readily understandable when one has the fresh specimens in hand. These always occur on the thicker, fleshier parts of the sheaths. These parts mature more slowly than the margins, thus simulating the conditions obtaining in the longer-growing tissues of the leaf blade where aecia normally develop from this primary infection, as noted below.

The infection on the sepals is not commonly observed because they soon drop off. If a search is made, however, at the right time, they can usually be found in considerable abundance on the ground among flowering stalks, and if infection is abundant in the patch on stems and sheath leaves these fallen sepals will almost invariably be covered with telia. Infected sepals still attached to the plant have, however, been found both at La Fayette and Ithaca (Pl. 1, A).

When basidiospore infection occurs on the blade of the leaf, aecial lesions almost invariably result. These show at first pycnia on the upper surface, followed shortly by the aecia below. It should be emphasized that the basidiospores which cause this infection may result from the germination of either the early or late crop of teliospores of the previous season.

The aecial lesions are rarely evident until after the teliospores on the sheath leaves, base of stem, and sepals have begun to mature. Fully developed aecia are seldom found earlier than a week after an abundance of mature teliospores appears on sheaths and stalks. It is obvious that these early telia could not have been produced from aecial infection. The delayed appearance of the aecia is perhaps due to the slower development of the fungus in the less rapidly maturing tissues of the leaf blade.

As the aecial lesions begin to mature, that is, when the cups are open and the spores are being discharged, one often finds, usually on the upper surface, about the margins of these lesions or more commonly on any larger veins which may be included in the lesion and even in the center of the spot among the pycnia, a rather abundant production of telial sori along with more or less development of epiphyllous aecia (Pls. 2 and 3). These telia are like those developed on sheath and stem and are certainly developed on the same mycelium as the aecia. A careful study made by one of the writers (Jackson) of the sori in such lesions has revealed the fact that occasionally a greater or less production of chains of aeciospores may be found in these telial sori. In similar situations aecia may occasionally be found with a few teliospores in the margin. In one specimen studied a sorus was found, one-half of which consisted of aeciospore chains and one-half of teliospores.

Occasionally aecial lesions may be found in which pycnia do not develop and quite commonly only a very few pycnia are found. When infection takes place on leaves which are relatively quite mature the number of telial sori formed may be greater than the number of aecia (Pl. 3, A and B). In one instance noted in the culture results, telia accompanied by pycnia, but with no aecia present, resulted from basidiospore infection on the leaves. Such a condition exactly parallels the normal result when infection occurs on the sheaths and stems, and is comparable to the usual condition of a micro-Puccinia.

It has been observed at Ithaca, N. Y. (Whetzel), that a very much smaller number of leaf blade infections resulting in aecia occur than of infections on scale leaves and stems resulting in telia. Patches of Podophyllum have often been noted in which the early crop of telia on sheaths and stems was almost universal, but in which no aecial lesions on the leaf blades developed during the season. In that locality no cases have been observed where more than a small percentage of plants showed aecial infections. This, however, is not the case at La Fayette, Ind. Aecial lesions on the leaves are usually found in considerable abundance in infected patches. It would be interesting to have data on this point from other sections of North America, particularly from regions at the northern limit of the range of the host species.

The aeciospores are scattered by the wind and cause infection of the surrounding expanded leaves of the Podophyllum plants. This infection is usually very profuse and general, and after a period of ten days or two weeks angular yellowish spots appear, on the under side of which are developed the gregarious, pulverulent telia bearing the second or summer crop of teliospores (Pl. 4, B). These mature and fall to the ground to mingle with those of the early crop, which have already been largely disseminated.

Reference has repeatedly been made to the two crops of teliospores. Those which appear early in the season on the sheaths, stems, sepals, and in association with the aecia on the leaves form the first crop, and those which arise from the infection of aeciospores form the second crop. There is, however,

A.—Upper surface of leaflet inoculated when quite mature with overwintered teliospores o late or summer crop. Note abundant production of telia and scanty infection

B.—Lower surface of same leaflet shown in A. Note development of telia almost to the exclusion of aecia

C.—Upper surface of leaflet inoculated when young with overwintered teliospores from the early crop on the stems. Note abundant production of telia among the pycnia and the vigorous infection

D.—Lower surface of leaflet shown in C. Note abundant development of aecia with an occasional telial sorus. Contrast with B

in addition to the difference in seasonal appearance, a noticeable difference in the character of these two sorts of telia, difficult to describe but easily recognizable when one has the specimens before him. In the early crop, the telia are usually somewhat larger, less definitely circular in outline, and somewhat longer covered by the cinereous epidermis. They also cause a slight but distinct hypertrophy of the host tissue similar to that produced by the aecia. When on succulent tissue they also appear to have their origin much deeper in the tissues. This difference is perhaps best brought out by comparing Plate 1, A and B with Plate 4, A and B.

It seems probable that this difference in type of sori is correlated with the fact that the early crop of telia on sheaths, stems, and sepals, as well as those which occur in association with the aecia, have a gametophytic origin, while the late crop which develops from aeciospore infection is sporophytic in origin.

The writers have been unable to obtain any evidence either from observation or cultures to show that this species possesses secondary or repeating aecia. Cultures conducted both in the field and in the greenhouse under control conditions have shown that the aeciospores give rise to the summer crop of telia.

SUMMARY OF LIFE HISTORY

The teliospores of both the early and late crops are functionally and morphologically indistinguishable and the basidiospores developed on the promycelium of either sort may, after overwintering, cause infection on any exposed portion of the Podophyllum plant. When infection takes place on the sheath leaves, stems, or sepals, telia are at once produced. They may or may not be accompanied by a few pycnia or aecia. When, on the other hand, infection takes place on the blade of the leaf, pycnia followed by aecia are developed. Aecia may develop without accompanying pycnia. Telia similar to those on sheaths and stems may or may not accompany the aecia on the leaves, and when present develop from the same mycelium as the aecia.

The late or summer crop of telia develops from aeciospore infection. There is no evidence of repeating aecia.

GENERAL DISCUSSION

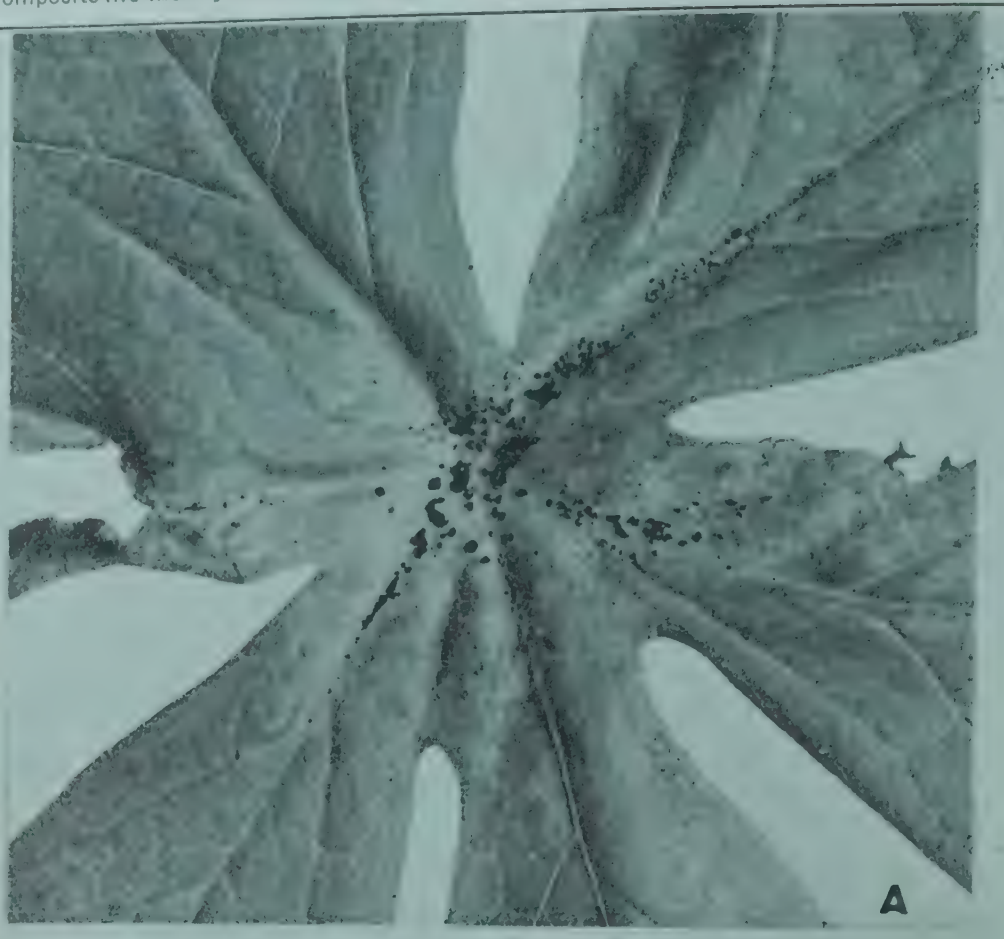
It is evident from the above that the writers' interpretation of the life history differs radically from that of Olive. It now becomes necessary to attempt to correlate this new interpretation with Olive's cytological observations. The writers have at present no basis for criticizing these observations nor do they see any reason, for purposes of discussion, for not accepting them as matters of fact.

It must be admitted, however, that in view of the experimental results recorded above, it would be highly desirable that the cytological situation in *Puccinia podophylli* be reinvestigated. It is apparent that we are dealing with a rust showing considerable plasticity in its development since from basidiospore infection teliospores are the first spore form produced under some conditions, while under others, pycnia followed by aecia occur.

It will be recalled that Olive found a small amount of uninucleate mycelium with the developing telial sori on the sheaths and an abundance of such mycelium in lesions on the leaves, but that the aeciospores as well as the teliospores developed on a binucleate mycelium. No so-called sexual fusions were observed to take place at the base of the aecial cups. It was assumed that all the telia and aecia were formed on a perennial binucleate mycelium and the pycnia were formed on a perennial uninucleate mycelium. The aecia were then assumed to be secondary in nature and sexual fusions would not be expected. They were to be expected, according to this view, in the primary aecia which would originate from basidiospore infection. These Olive assumes he had not seen, and evidently considered it doubtful whether they would be found to be present in the life history.

In view of the evidence already presented it would hardly seem necessary to consider seriously the possibility of a systemic perennial mycelium in the case of this parasite. However, the paper by Olive has given this theory such general acceptance that it seems necessary to emphasize the fact that the writers have not been able to obtain the slightest evidence that the rust is in any sense perennial, or even systemic, in the host plant.

According to the view of the writers, accepting the cytological findings of



A.—Upper surface of leaf showing aecial lesion which involves the disk and the veins. Note the pycnia and the abundant development of telia. Natural infection

B.—The late or summer crop of telia on upper and lower surface of leaves. Compare with A and with Plate 1, A and B. Natural infection

Olive, the conjugate condition may be assumed to arise on the mycelium before the primordium of the sorus develops, or at least in a very early stage of its development. In connection with the sori on the sheaths, stems, and sepals, the change from a uniucleate to a binucleate condition would be expected to take place soon after infection, which would account for Olive having found uninucleate mycelium only in small amount. On the leaves, however, the change would be expected to take place later, after the pycnia are formed, and hence, as Olive found to be the case, a considerable mass of uninucleate mycelium would be expected.

Once the binucleate condition has been established in the mycelium there are no difficulties from a cytological point of view as to the spore form produced, since both aeciospores and teliospores arise from binucleate cells. It is very likely that the type of spore produced is determined by the nutritional conditions prevailing in the host. However, it should be borne in mind that the tendency to an unstable condition is quite certainly inherent in the rust itself and the nutritional factors are best interpreted as secondary, serving primarily to explain the situation as we find it and in view of our present, perhaps incomplete, cytological knowledge.

That telia are the first structures developed from infections on the sheaths, stems, and sepals is perhaps correlated with the influence of the rapid maturity of the tissues of these organs and the nature of the available nutrient. This influence was recognized by Olive. He used it, however, to explain why telia were formed instead of "secondary" aecia on the "perennial" sporophytic mycelium.

The occurrence of telia in association with the aecia on the leaf blades, which has been repeatedly mentioned, is readily correlated with Olive's cytological observations and can best be explained also by the factor of food relations. Olive finds that the aecia are borne on a binucleate mycelium. It would appear quite logical to expect that this same mycelium might produce telia when the factors which determine teliospore production become operative.

The development of telia in association with aecia usually occurs sometime after the majority of the aecia are mature. It would seem reasonable to suggest that the development of telia under these conditions may be due to the depletion of the local food supply by the mycelium during the development of the aecia and aeciospores, or directly to the more mature condition of the tissues. Perhaps, however, their formation is best explained by a combination of these two influences. In some cases the telia that develop in association with the aecia appear practically simultaneously with them. This is especially true when the lesion involves the midrib or one of the larger veins. In such cases the explanation is similar to that given above to account for the development of telia only on stems, sheaths, and sepals. The tissues of the veins and midrib are quite comparable in the character of the food supply to that of the stem. It has already been noted in the discussion of the life history that when mature leaves are infected with basisiospores, telia may predominate over the aecia in the resulting lesions and in rare cases may be formed exclusively.

It has been repeatedly observed in connection with many long-cycle rusts, that as the hosts approach maturity there is a gradual reduction in the development of repeating spores and a corresponding increase in the development of the teliospores either in the same or separate sori. It would seem that this situation exactly parallels that in the Podophyllum rust except that since there are no repeating spores the teliospores developed in association with the aecia normally occur in separate sori. As noted in the discussion of the life history, however, teliospores may rarely develop in the aecial cups.

It is important to note, however, that only a few other cases have hitherto been observed where aecia have been replaced by telia. In most rusts the maturity of the tissues affects aeciospore production largely by reducing the number of aecia produced or the quantity of aeciospores. It has been repeatedly observed in the laboratory at La Fayette in connection with heteroecious culture work that when over-mature leaves or those in a poor growing condition are inoculated, while pycnia may be formed to some extent, few if any aecia develop. The fact that aeciospores are replaced by teliospores in the Podophyllum rust is therefore another indication of the plastic and unstable condition of this species.

The assumption that the conjugate condition arises at an early stage in the development of the mycelium can also be used to explain the fact that aecia may occasionally be developed without the pycnia. In such cases the change may have taken place

very early, before a sufficient mass of mycelium has been formed to enable the pycnia to develop. Either aecia or telia might then develop on the mycelium as the first spore form following infection, dependent on the available food supply or the maturity of the tissues.

It is well to recall at this point that the telial sori on stems, sheaths, and sepals strongly suggest the normal situation in microforms, and it is enlightening to review briefly the cytological situation which has been found in the species of short-cycle Puccinia and Uromyces which have thus far been studied.

It is convenient to consider these in two groups. In the first the primordium of the telium is made up of uninucleate hyphae, and the conjugate condition arises as a definite process, by cell fusion or nuclear migration, in a manner similar to that found in aecia. This is the general situation in the following species: *Puccinia* (*Nephlyctis*) *transformans* Ellis and Ev. investigated by Olive (*5*), *Puccinia* (*Polythelis*) *fusca* (Pers.) Wint. by Pavolini (*7*), *P. malvacearum* Bertero by Werth and Ludwigs (*8*), *P. buxi* DC. by Moreau (*4*) and *Uromyces scutellatus* (Schrank) Lev. and *Puccinia rossiana* (Sacc.) Lagh. by Kursanov (*3*). *Uromyces laevis* Körn., studied by Kursanov, probably also belongs in this group, but the actual process of the formation of the conjugate condition has not been observed.

In the second group the primordium of the telium is made up of binucleate hyphae and the vegetative mycelium is also primarily binucleate. The transition from a uninucleate to a binucleate condition in these forms has unfortunately not been observed, but it is assumed by the authors that it occurs very early in the vegetative growth of the mycelium. This group includes *Puccinia fergussoni* Berk. & Br., *P. asarina* Kze., *P. aegopodii* (Schum.) Mart., *Puccinia* sp. (*P. conferta* Diet. & Holw.?) on Artemesia, and *Uromyces gagae* Berk. as investigated by Kursanov (*3*) and *P. adoxae* Fuck. and *Uromyces scillarum* (Grev.) Wint. by Blackman and Fraser (*2*).

It should be pointed out that, except for *Puccinia malvacearum*, *P. buxi*, and *P. rossiana*, the species of the first group normally develop pycnia; while in those of the second group, without exception, pycnia are normally absent.

Uromyces ficariae Wint. is especially interesting in this connection. Blackman and Fraser (*2*) record that—

> the general mycelium appears to exhibit single nuclei, but the mass of mycelial hyphae round about the teleutospore sorus as well as those directly connected with teliospore formation appear to have conjugate nuclei.

They did not find how or where the change took place. Moreau (*4*), however, found all the mycelium uninucleate and the binucleate phase beginning in lower cells of the telial primordium. Kursanov (*3*), on the other hand, more nearly confirms the findings of Blackman and Fraser (*2*). He finds, however, only a small amount of uninucleate hyphae, the vegetative mycelium being prevailingly binucleate.

A more detailed review of the literature of this subject would show that in the first group there was considerable variation in the amount of sporophytic tissue developed between the origin of the binucleate condition and the formation of teliospores.

We apparently have, therefore, in some forms of short-cycle rusts a very short sporophyte, while in others the origin of the conjugate condition has arisen at varying distances back from the mother cell of the teliospores, sometimes only a little way, sometimes far, possibly even near to the point of infection by the gametophytic basidiospore.

It seems to the writers that this cytological situation, especially as found in the second group of species mentioned above, parallels very closely that described for *P. podophylli* by Olive. It is unfortunate that in these short-cycle forms the fusions have been observed only in the cases where the change takes place at the base of the telium. This circumstance, however, serves to point out the wealth of problems awaiting solution in this field, and it is hoped that students of cytology will soon give us the necessary link to make the chain of evidence complete.

PHYLOGENETIC CONSIDERATIONS

It is pertinent to ask what is the significance of the peculiar life history as shown by the Podophyllum rust. Without attempting at this time to go into the evidence which has determined their position, it may be stated that the writers of this paper are among those who believe that, regardless of what the most primitive rust may have been, the long-cycle species in the group Pucciniaceae at least are the older and that the short-cycle forms as exemplified by the microforms of Puccinia and Uromyces are derived or reduced forms and are relatively more recent.

It follows, then, that during the process of development from a long to

a shorter type of life history some intergrading conditions are to be expected. It is not at all surprising also to find evidences of a profound readjustment and an apparently unstable condition in the nuclear phenomena in some forms.

The writers believe that *Puccinia podophylli* is a form which still exhibits evidences of the sort of changes which may have taken place in the development of the reduced forms from the more complex.

SUMMARY

1. *Puccinia podophylli* shows no evidence of being perennial or systemic in the host.

2. The early crop of teliospores which occur on the bud scales, stems, and sepals preceding the aecia arise directly from mycelium produced by basidiospores from overwintered teliospores and are usually not accompanied by pycnia.

3. The aecia which normally develop on the blade of the leaf also arise in a similar manner from the same source. Pycnia are usually found associated.

4. The late or summer crop of telia are produced on mycelium developed from infection by aeciospores.

5. There is no evidence of repeating aecia in this species.

6. Basidiospores from either the early or late crop of teliospores may result in the production of either the early telia or the aecia.

7. Telia may develop in association with the aecial lesions and arise directly from the same mycelium.

8. When mature leaves are infected telia may predominate over the aecia, with or without the development of pycnia.

9. *Puccinia podophylli* exhibits evidences of being in an unstable or plastic condition as to life history.

10. It is suggested that the food conditions of the various tissues invaded have an important influence on the spore form developed.

11. This species is believed to be a form which still exhibits evidences of the sort of changes which may take place in the evolutionary development from the complex to the simpler forms of life history.

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EFFECT OF HEIGHT OF CHIPPING ON OLEORESIN PRODUCTION [1]

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INTRODUCTION

"Gum," as the oleoresin from the living slash and longleaf pine trees of the southern United States is called, is obtained by cutting a gash or "streak" across the grain of the outer sapwood at the butt of the tree. The depth into the tree to which this cut extends and the height of the chip of wood removed have varied in commercial practice, and still vary widely. A new cut has to be made, generally at weekly intervals, to freshen the exposed surface and secure a flow of gum. The successive cuts or chippings, one close above the other, constitute the "face." (See Pl. 1.) During a season's work, generally between March and December, about 32 chippings or streaks are cut.

The objection of some timber owners to turpentining virgin timber is due to the very injurious methods of chipping which have prevailed all too widely. A large amount of timber has been killed, injured, or at least its grade unnecessarily lowered, in this way.

For some time past the Forest Service of the United States Department of Agriculture has been testing operating methods for the purpose of determining ways of improving turpentine production and at the same time of conserving timber. The administration of the Florida National Forest has indeed demonstrated over a considerable period and on a commercial scale the beneficial results that can be obtained on mature longleaf pine timber by the use of a streak which removes a chip ½ inch deep and ½ inch high each week,[2] a more conservative practice than that obtaining in many private operations. Tests were also started in 1923 and are now under way at the substation of the Southern Forest Experiment Station at Starke, Florida, to determine the effect of different carefully controlled methods of chipping on second-growth slash and longleaf pine.[3] The test reported here is one of still another series[4] which has been conducted for a number of years by Dr. Austin Cary, logging engineer (Branch of Forest Management), in which the writer and, in this case, Deputy Supervisor E. R. McKee and W. H. Graham, commercial turpentine operator on the Florida National Forest, also cooperated. Both in tests and in commercial turpentining many indications have pointed to the fact that removing less wood produces conditions in the tree which favor high sustained production.[5] "Low" chipping permits a reduction in the size of the scar cut each year, thus lessening the amount of possible degrade. It also lengthens the time during which a given crop may be operated, if so desired.

Therefore, since the results from "low" chipping indicated decided operating advantages, but since no data on directly comparable stands were available over a sufficiently long period to be conclusive, the turpentining test described herein was instituted. It was conducted in connection with an actual commercial operation, the chipping being done by the regular worker assigned to the tract, and the gum, after being weighed, was collected and stilled together with the other gum

[1] Received for publication July 23, 1924. A report of a test made by the Forest Service in 1923 to determine the effect of different heights of chipping in turpentining longleaf pines.

[2] McKEE, E. R. NAVAL STORES PRODUCTION ON THE FLORIDA NATIONAL FOREST. Naval Stores Rev. 33 (6): 16–17; (7) 6, 23; (8) 6, 19; (9) 10, 23; (10) 16–17. 1923.

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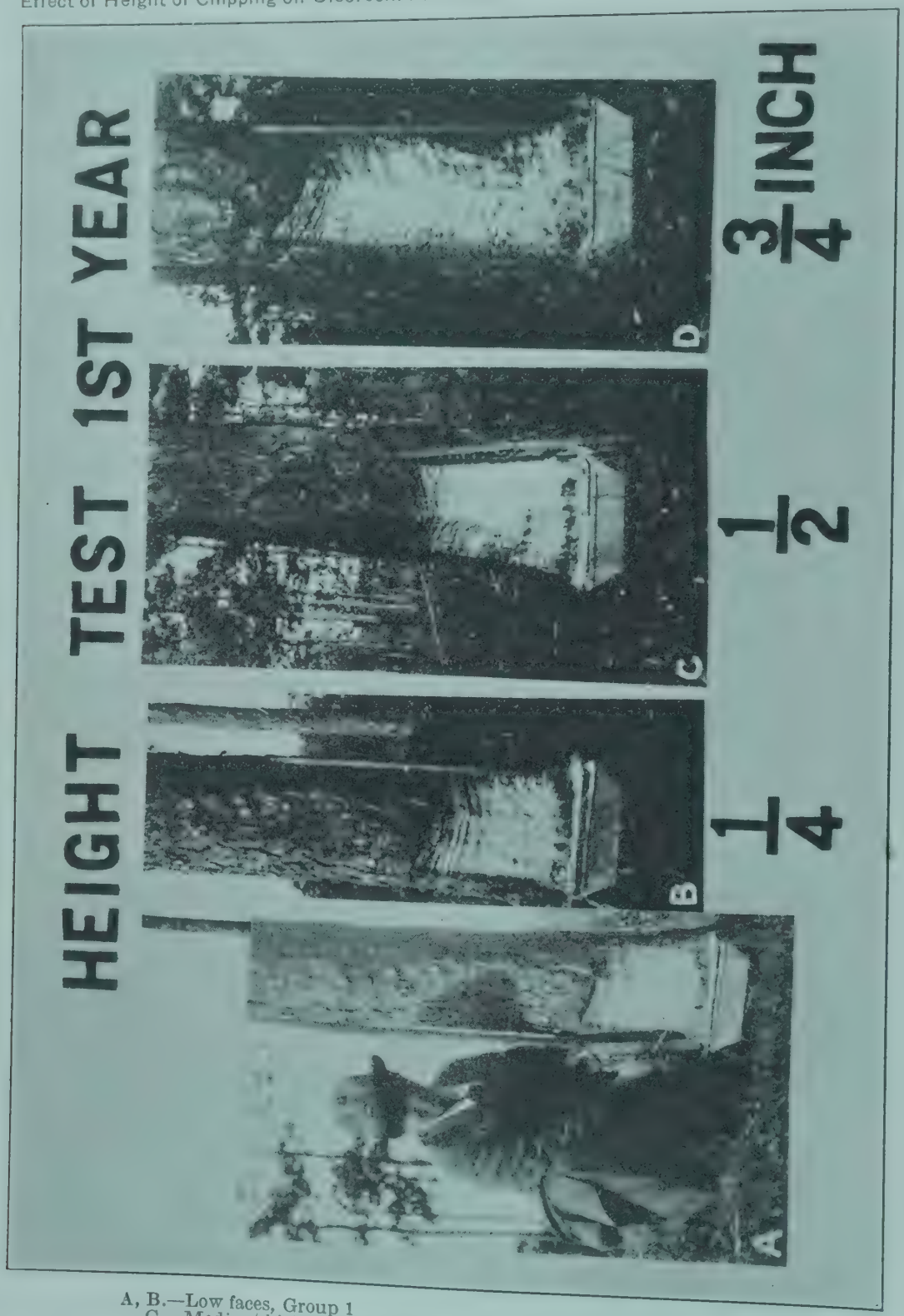
[5] GERRY, E. OLEORESIN PRODUCTION. U. S. Dept. Agr. Bul. 1064, 46 p., illus. 1922. ——— RECENT OBSERVATIONS ON THE EFFECTS OF TURPENTINING ON THE STRUCTURE OF SECOND-GROWTH SLASH AND LONGLEAF PINES. Jour. Forestry 21: 236–241, illus. 1923.



A, B.—Low faces, Group 1
C.—Medium high faces, Group 2. Standard Forest Service practice
D.—High faces, Group 3. Common commercial practice

obtained from the area. The test probably will be continued until 1927. Similar tests will be conducted by Dr. Cary and the writer in cooperation with other interested operators. Various types of slash and longleaf pine timber in widely scattered localities in the South are to be studied. Practically stated, the object of this investigation, of which the test described below is a part, is to determine whether there is any good reason why operators should not cut "lower" faces; that is, remove at each cutting less wood than is now customary.

DESCRIPTION OF THE TEST

CHARACTER OF TREES SELECTED

The 60 longleaf pine test trees constituting three matched groups are located near a main road in the Florida National Forest and are easily accessible from the Ranger Station at Camp Pinchot, Valparaiso, Fla. The tract in which they stand had been set aside to be worked commercially for turpentine, beginning in 1923. Conditions of soil and general site apparently are comparable for all. The individual trees were matched by diameter measurement breast high and by visual inspection for similar external features, such as crown density, character of bark, and absence of wounds or fire scars.

It is realized that 60 trees is a small number. The results and conclusions drawn are, consequently, to be considered as indicative only. The helpfulness of these small tests, however, has been clearly demonstrated. They offer opportunities for careful detailed selection and matching of individual trees which larger ones do not, so that they may be compared as closely as possible. This was true in the test described, where both external features and internal structure run parallel to a fairly satisfactory degree in the groups.

The test trees were divided into three similar plots or groups of 20 each, to be chipped at different heights. They were distinguished by suitable markings, that is, one spot of white paint on the bark of each tree in Group 1 and two and three spots, respectively, for each tree of Groups 2 and 3. The average diameter breast high for the three groups was practically the same (11.5 inches), with individual variations ranging between 9 and 14 inches. The number of annual rings in the half inch of wood next the bark, counted on chips removed during the turpentining, showed group averages of 15.6, 14.6, and 18 rings, respectively. Groups 1 and 2 were selected on November 24, 1922. The third group, representing the commercial practice of many operators, was added for observation on April 9, 1923.

A view showing some of the Group 1 trees which are generally typical for the stand in the test is shown in Plate 3. The timber is not young second-growth. It is generally uniform in character, typical of much that is now being worked, but is not to be classed with fast-growing young longleaf pine on better soil. Neither is it like some of the old, over-mature trees which may be found in this locality. It presents a healthy external appearance and a normal wood formation, as is indicated by the number of rings per inch, shown in the accompanying photomicrographs (Pl. 2).

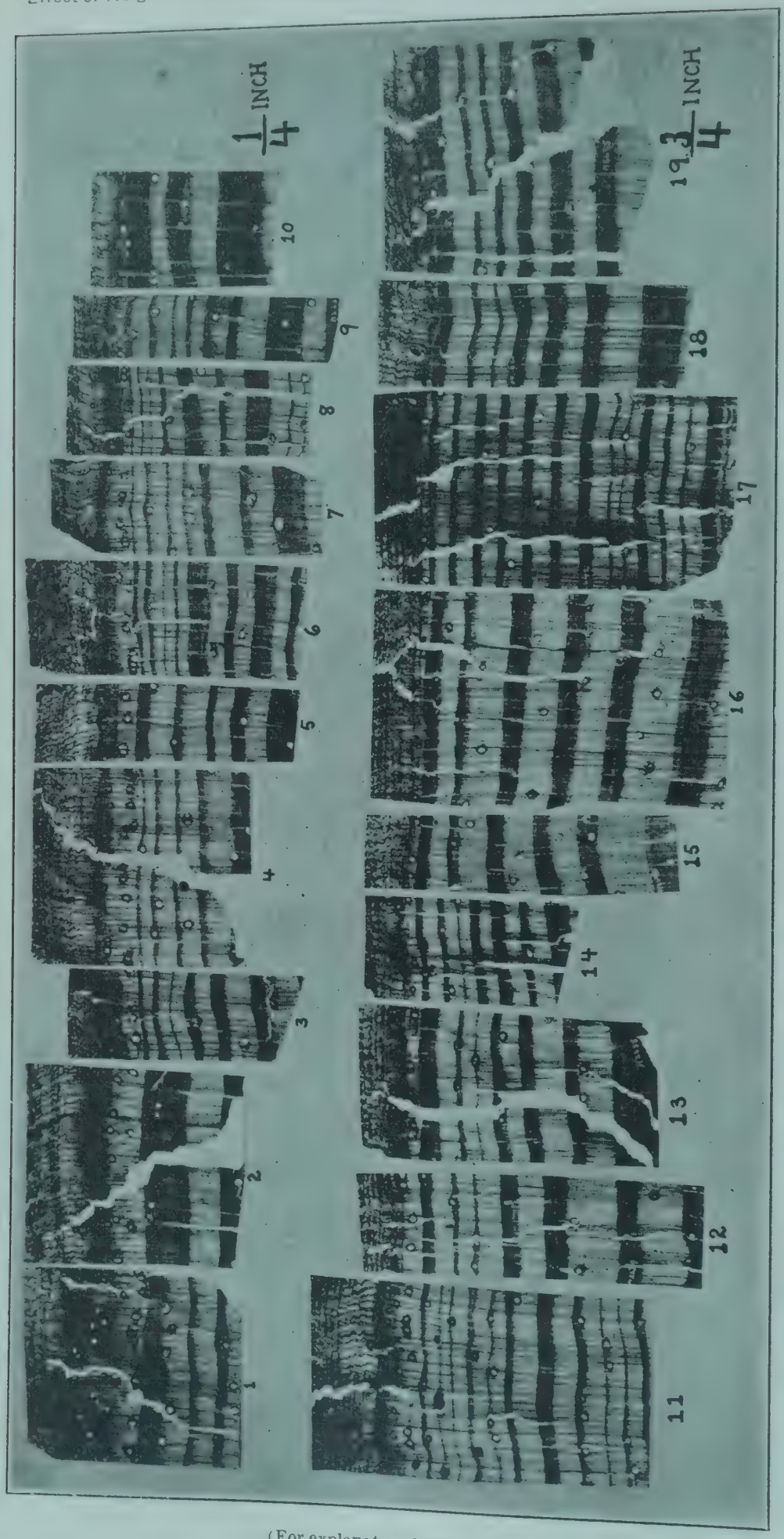
METHODS OF CHIPPING

HISTORY OF FIRST YEAR'S WORK.—The usual advance streak was cut on all trees three or four weeks before the regular chipping, which began on February 17, 1923.

Because of the Deputy Supervisor's illness, the first six streaks were cut without special supervision, ½ inch high, according to the standard practice of the Forest Service, and comparative test chipping was not regularly begun until April 9. Consequently, on that date the faces were an inch and a half higher than was intended for Group 1 and lower by the same distance for Group 3. It chanced, moreover, in surveying the commercial crop[6] lines through the tract that the trees situated in Group 2 were included in a separate crop from the other test trees, so that at first they were chipped by a different workman and later in the week. This would tend to make the yield at the first dipping appear unduly low for Group 2. After June 1 it was arranged that all trees should be chipped on the same day by the same man. Collection of gum (dipping) occurred at the intervals noted in Table I.

The faces are all placed low on the trees, as is the regular practice on the Forest, with the first streak 7 inches or less from the ground. Regular chipping for the year stopped about November 8, 1923, at 32 streaks, but a thirty-third streak was cut to obtain material for microscopic study. At the end of the season the faces were scraped. The yields of gum and

[6] A "crop" is approximately 10,000 faces, or about the number that one man will chip in a week.



(For explanatory legend see p. 85)

"scrape" obtained are shown by weight in Table II.

HACK USED.—Chipping was done with a No. "0" hack, a tool smaller than that generally used. With this it is easier to cut a light chip.

DEPTH OF CHIPPING.—All 60 trees were chipped to the same depth, nominally one-half inch, which has been found successful with this type of timber.

HEIGHTS OF CHIPPING COMPARED.—On the 20 trees in Group 1 a chip as nearly as possible ¼ inch high was removed each week. This is much less wood (in the vertical direction) than is now removed in ordinary commercial chipping, though frequently a chip no higher than this will be removed when the faces are high and a "puller"[7] has to be used.

The 20 trees in Group 2 were chipped ½ inch high each week, according to the standard practice of the Forest. So skilled have the chippers on this forest become that when using an "0" hack they easily and accurately carry on this light chipping and even tend to remove a chip slightly less than ½ inch high. Such chipping has already been found to produce desirable results on a commercial scale.[8] The Group 2 test chipping averaged 0.45 inch for the 1923 season. (See Table I.)

On the 20 trees in Group 3, "high" chipping, ¾ inch per week, was practiced. This corresponds to work now being done in many commercial operations.

WIDTH OF FACE.—The average width of the face at the end of the 1923 season was 9.6 inches for Groups 1 and 3 and 9.3 inches for Group 2.

With the overcoming and adjusting of the few slight difficulties and irregularities described above, the test progressed very satisfactorily to the end of the season, and has been resumed in 1924. In Plate 1 are shown typical faces as they appeared on November 24, 1923.

YIELD OF GUM IN RELATION TO HEIGHT OF CHIPPING

RESULTS FROM THE TEST

In Table II are recorded the weights of gum obtained from the three groups of trees during the 1923 season. The average yield per streak per tree at each dipping is exhibited graphically in Figure 1.

With the yield of Group 1 taken as a standard, Group 2 fell below it 8.4 per cent in amount of gum (dip) obtained and 7.2 per cent in amount of dip and scrape combined. Group 3 as compared with Group 1 was 10.5 per cent lower in dip but only 4.9 per cent lower in combined dip and scrape. With Group 2 (Forest Service practice) taken as a standard, Group 3 (common commercial practice) fell 2.5 per cent below in yield of dip; but if dip and scrape combined are considered, Group 3 was 2.7 per cent higher (for the first year) than Group 2.

TABLE I.—*Average height of faces desired and obtained in a chipping test on longleaf pine, Camp Pinchot, Fla., 1923 (measured on 32 streaks)*

Height of cut	Group 1	Group 2	Group 3
Total height face (inches):			
Contemplated	8.0	16.0	24.0
Actual	10.7	14.4	21.4
At test rate	[a] 9.2	14.4	[b] 22.9
Average height streak (inches):			
Asked	0.25	0.50	0.75
Obtained	0.28	0.45	0.71

[a] Actual height minus 1.5 inches extra height cut during first 6 weeks of chipping, when a streak ½ inch high was cut each week.

[b] Actual height plus 1.5 inches. During first 6 weeks of chipping the face was cut 6/4 inches too low since each streak was ½ instead of ¾ inches high.

[7] With the puller the cutting of the streak is done by the muscle power of the chipper, whereas with the hack the weight on the end of the handle makes it seem easier to cut the streak. This accounts for the fact that less wood is removed during pulling.

[8] MCKEE, E. R. OP CIT.

EXPLANATORY LEGEND FOR PLATE 2

Cross sections of chips cut Nov. 24, 1923 (end of season), from Groups 1 and 3. Longest sections show about ½ inch of wood.

Top row.—Chips from low faces, Group 1 trees. Note (1) wide 1923 annual ring next bark, at top, (2) with numerous resin passages; (3) most of the 1923 rings as wide as or wider than those of 1922.

Bottom row.—Chips from high faces, Group 3 trees. Note (1) narrow 1923 annual ring next bark, (2) with few resin passages; (3) 1923 annual ring often narrower than that of 1922.

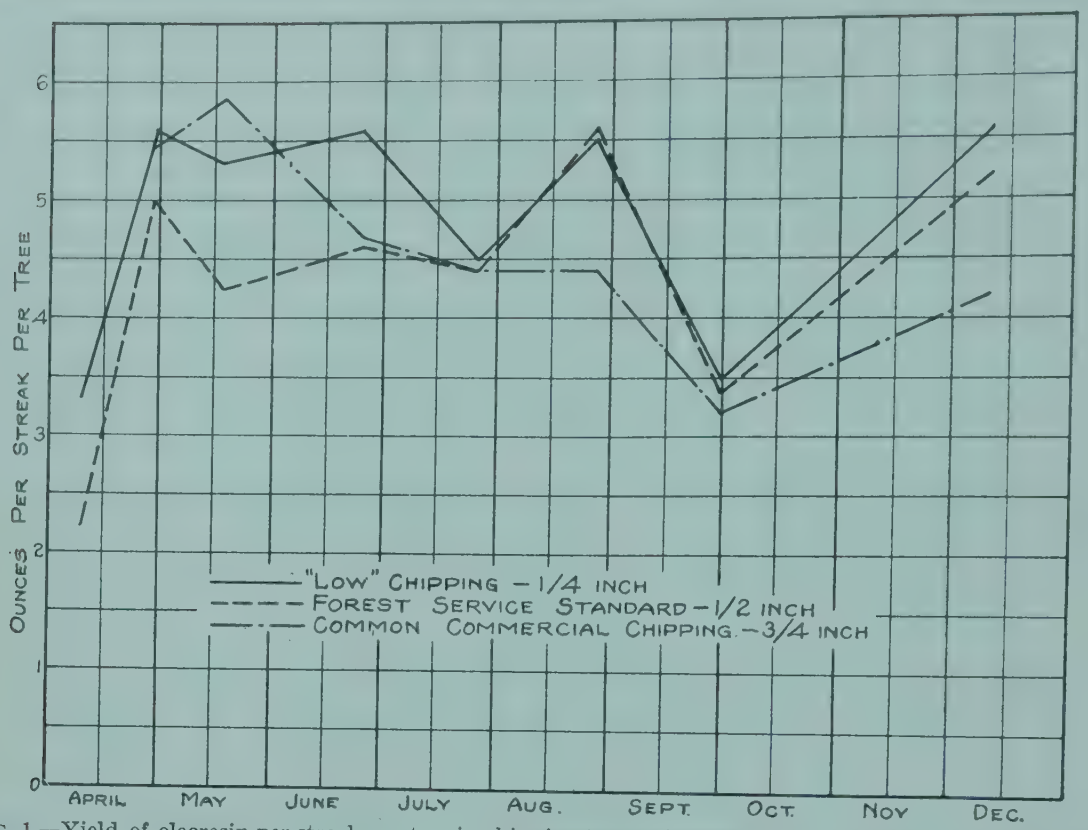


FIG. 1.—Yield of oleoresin per streak per tree in chipping test on longleaf pine, Camp Pinchot, Fla., 1923

TABLE II.—*Yields of oleoresin obtained in chipping test on longleaf pine, Camp Pinchot, Fla., 1923*

Date of dipping	Number of streaks cut since last dip	Yields: Group 1 (¼-inch chipping) Total for 20 trees	Group 1 (¼-inch chipping) Per streak per tree	Group 2 (½-inch chipping) Total for 20 trees	Group 2 (½-inch chipping) Per streak per tree	Group 3 (¾-inch chipping) Total for 20 trees	Group 3 (¾-inch chipping) Per streak per tree
		Pounds	*Ounces*	*Pounds*	*Ounces*	*Pounds*	*Ounces*
Apr. 9 [a]	6	25.00	3.33	[b] 16.75	2.23	[c]	
Apr. 28	3	21.00	5.60	18.75	5.00	20.50	5.46
May 18	3	20.00	5.33	16.00	4.26	22.00	5.86
June 25	4	28.00	5.60	23.00	4.60	23.50	4.70
July 26	4	22.50	4.50	22.00	4.40	22.00	4.40
Aug. 27	4	27.50	5.50	28.00	5.60	22.00	4.40
Oct. 1	5	22.00	3.52	21.00	3.36	20.00	3.20
Dec. 10	4	28.00	5.60	26.00	5.20	21.00	4.20
Total dip for season	33	194.00	5.01 (0.313 pound)	171.50	4.59 (0.287 pound)	--------	4.48 (0.280 pound)
Comparable total dip for season	27	169.00	----------	154.75	----------	151.00	----------
Total scrape	--------	43.00	----------	42.00	----------	51.00	----------
Total comparable dip and scrape	--------	212.00	----------	196.75	----------	202.00	----------

[a] Dipping of April 9 not counted in comparable total because Group 3 was lacking.
[b] Chipped later in week than Group 1; full amount of gum not in cup at first dipping.
[c] Weight not taken.

The fact that the ¼-inch chipping gave a high total yield of gum and produced a particularly high yield during the latter part of the season is of great significance if found to be maintained in later years and confirmed by other tests. This is not the first time that such a result has

been clearly indicated. The fact that as much gum as usual, or even more, was obtained in this test from trees which had considerably less than half as large a scar as is frequently used is worthy of note. Not only is it a matter of scientific significance, the causes of which are to a considerable extent explained by studies of the microscopic structure of the wood, but it is a matter of practical importance as well. By lightening chipping an operator is at no additional expense; he is also, if this test is typical, obtaining at least as high a yield as with the old methods. Furthermore, he is keeping his trees more healthy and productive because reducing the size of the wound, and, lastly, he is reducing the source of possible lumber degrading due to pitch-soaking behind the face.

Additional data from this test and also results from "low" chipping experiments at other points will be available at the end of the 1924 season.

CALCULATION OF YIELDS ON A CROP BASIS

The yield from the last 27 streaks, those cut according to test requirements on the 20 selected trees in each group, converted to a yield per crop (10,000 cup) basis, is shown in Table III. In the test-yield figures the omission of the amount of gum obtained from the first 6 streaks to some extent compensates for the fact that the crop calculation is based on the yield of selected trees. Since the calculated figures may still be a little high, the possible excess has been further allowed for herein below by an arbitrary reduction in the estimated value of the products. The difference in response of the three groups appears to be a real one, as is shown in the microscopic data presented later.

For comparison, the average results of working trees on a commercial operation were obtained for the tract in which the test trees stand. Here 33,521 cups were included in a first-year working by methods the same as those employed in test Group 2. The yield [9] obtained was 618.4 barrels of gum and 212 barrels of gum and scrape mixed. The latter produced about 8 gallons of spirits of turpentine per barrel and the former about 12. Hence the total yield was approximately 9,116.8 gallons, or 182.33 barrels, of turpentine. The number of cups worked was equivalent to about 3.35 crops, so that the yield per crop (10,000 cups) averaged about 54.4 barrels of spirits. By calculation of the Group 2 yields, without any deduction, a production at the rate of about 60 barrels would be indicated. This, however, presupposes vigorous, healthy, comparatively young trees, which probably average higher in yield than many of the over-mature, old-growth trees which are of necessity included in the commercial working.

TABLE III.—*Conversion of test (20 cup) yield to a crop (10,000 cup) yield basis Chipping test on longleaf pine, Camp Pinchot, Fla., 1923*

	Group 1	Group 2	Group 3
Yield from gum:			
1. Actual net yield of gum from 20 trees (Apr. 9 to Dec. 10, 1923; first 6 weeks omitted)—pounds	169.00	154.75	151.00
2. Calculated net yield of gum for 10,000 cups—do	84,500.00	77,375.00	75,500.00
3. Calculated number of barrels of gum (net weight 410.5 pounds each)—barrels	205.85	188.49	183.92
4. Calculated quantity of turpentine (one barrel of gum yields about 12 gallons spirits)—gallons	2,470.20	2,261.88	2,207.04
5. Value at 98 cents per gallon—dollars	2,420.80	2,216.64	2,162.90
6. Number of barrels of turpentine (50 gallons each)—barrels	49.40	45.24	44.14
Yield from scrape:			
7. Actual net yield of scrape from 20 trees—pounds	43.00	42.00	51.00
8. Calculated net yield for 10,000 faces—do	21,500.00	21,000.00	25,500.00
9. Calculated number of barrels (net weight 310 pounds each)—barrels	69.35	67.74	82.26
10. Calculated quantity of turpentine (one barrel yields about 5 gallons spirits)—gallons	346.75	338.70	411.30
11. Value at 98 cents per gallon—dollars	339.82	331.93	403.07
12. Quantity of turpentine (50 gallons per barrel)—barrels	6.94	6.77	8.23
Calculated total yield, gum and scrape combined:			
13. Total quantity of turpentine per crop. (Selected trees, 27 streaks) (6+12)—barrels	56.34	52.01	52.37
14. Quantity of turpentine from commercial operation (all trees, 32 streaks)—barrels		54.42	
15. Total value of spirits (5+11)—dollars	2,760.62	2,548.57	2,565.97
16. Approximate total amount of rosin (No. barrels turpentine×3)—barrels	169.02	156.03	157.11
17. Value of rosin at $4.80 per barrel—dollars	811.30	748.94	754.13
18. Total value of turpentine and rosin per crop (15+17)—do	3,571.92	3,297.51	3,320.10

[9] From information furnished through courtesy of the administration of the Florida National Forest.

FINANCIAL ADVANTAGES FROM LOW FACES

Taking the average price of turpentine as 98 cents a gallon and of rosin as $4.80 a barrel,[10] it has been shown in Table III that there would be a difference in value of the products between the "low" or ¼-inch chipping and the "high" or ¾-inch, amounting to more than $250 per crop; or to be very conservative, since the calculated yield may still be higher than an actual one based on a large number of variously conditioned trees, say, in round numbers, about $200.

Before undertaking the test there was no clear indication of an actual gain in total yield to be had the *first* year. Results on a large number of less carefully matched trees had even indicated a lower first-year yield than from trees more heavily worked. The advantage of increased yield during *later years* was, however, expected, and the next three years will show whether or not the expectation was justified. The advantages of reduced size of scar and, consequently, of better sustained vitality in the tree, more prolonged working, and reduced degrading of the butt lumber were obviously assured from the start. These will remain significant whether the yields during the first year are to be slightly less, the same as, or greater than those obtained from heavier work.

MICROSCOPIC STUDY OF WOOD FORMATION IN THE TEST TREES

MATERIAL

The progress of wood formation above the face scar on the test trees was followed by means of a microscopic study of chips collected on June 6, July 26, October 19, and November 24, 1923, at the height of the face on those respective dates. An examination of increment borings made on the Group 1 and Group 2 trees when they were first selected had revealed no significant differences.

RESULTS OF MICROSCOPIC STUDY

It has been found as the result of extensive study and many painstaking comparisons between the structure of the new wood formed after turpentining (especially that midway of the streak just above the face) and the yields and behavior of the trees worked that chips such as those obtained serve as very reliable indicators.[11] They show whether or not a tree has its vitality so impaired that it is incapable of producing an adequate return. They show finer points also, which are made clear by a comparison with the responses of the round timber similarly situated during the same year. The degree of response in such matters as width of annual ring, percentage of summerwood, date of beginning of wood formation in the spring, time of summerwood formation, and amount and character of resiniferous tissue (duct) formation are closely coordinated with the method of turpentining. These indications can often be obtained, by microscopic examination, in time to save undue injury to the timber as a result of too heavy working.

MIDSEASON EXAMINATION.—Microscopic characteristics of the July 26 chips, representing the midseason condition of the trees, are recorded in Table IV, as are also the characteristics of the chips taken at the end of the season, November 24.

It is obvious that by July 26 a considerable portion of the wood of the 1923 ring had been formed. In more than half the trees in Group 1 as many wood cells were already present as were found in the entire ring formed during the preceding year (1922). In Group 3 this was true of only about one-third of the trees. On the average, the trees chipped ¼ inch each week (Group 1) had wider rings, more summerwood cells, and more resin passages than those of the two other groups. This seems to indicate an earlier and more vigorous response in the Group 1 trees. Indeed, at this time they had produced on the average nearly three times as many summerwood cells and about twice as many resin passages as the Group 3 trees. Presumably because of the low chipping and removal of little wood, the trees in Group 1 were able to make a better adjustment to the exploitation of their energies for gum production. This notable structural difference was approximately concurrent with the difference in yield of gum, which was particularly apparent for Groups 1 and 3 from about this time to the end of the season, as is shown by the graph, Figure 1. It further emphasizes the

[10] Based on figures for the 1923–24 season by SPEH, C. AVERAGE MONTHLY SAVANNAH MARKETS FOR SEASON 1923–24. Naval Stores Rev. 34 (2):17. 1924.

[11] GERRY, E. OLEORESIN PRODUCTION. U. S. Dept. Agr. Bul. 1064, 46 p., illus. 1922. ——— RECENT OBSERVATIONS ON THE EFFECTS OF TURPENTINING ON THE STRUCTURE OF SECOND-GROWTH SLASH AND LONG-LEAF PINES. Jour. Forestry 21: 236–241. illus 1923

relation between gum production, vitality, and number of resin passages present. In Group 1, besides the normal number of resin passages in the old rings, these relatively more abundant extra wound ducts were, as is apparent from the plates and Table IV, already formed and active by midseason. In Group 3 only about half as many were functioning at that time. Table IV clearly shows that the effects of different methods of working made a progressive series. The poorest formation of wood and resin tissue occurred in the Group 3 trees; an intermediate but good condition was found in Group 2, but the best response was made by the Group 1 trees with the lowest faces.

after regular chipping had stopped, a thirty-third streak was cut on the test trees in order to obtain a comparable set of fresh chips on which to study the effect of the season's work at a point midway of the streak at the top of the face. From Table IV it will be clear that a similar, though less marked, relation to that found at midseason still existed between the groups. The average width of the 1923 ring, the amount of summerwood, and the number of resin passages was greater at this time in the Group 1 trees than in those of Group 3. There was, it is true, a slight increase in the average total number of resin passages found for Group 3 in November above that present in July, but their number was

TABLE IV.—*Comparative production of wood and resiniferous tissue in height of chipping test on longleaf pine, Camp Pinchot, Fla., 1923*

	Average width of 1923 ring			Average number of resin passages in unit area,[a] per year		Number of observations in which width of 1923 ring was found equal to, less, or greater than width of 1922 ring		
	Springwood cells	Summerwood cells	Total cells	1923	Average for 3 years before turpentining	Equal or nearly so	Less	Greater
Chips taken July 26, 1923:						*Per cent*	*Per cent*	*Per cent*
Group 1 (¼ inch)	7.8	18.3	26.1	12.0	1.10	31.8	31.8	36.4
Group 2 (½ inch)	11.0	12.9	23.9	9.0	1.08	5.0	40.0	55.0
Group 3 (¾ inch)	7.8	6.4	14.2	6.5	1.00	25.0	62.5	12.5
Chips taken Nov. 24, 1923:								
Group 1 (¼ inch)	12.6	19.2	31.8	11.4	1.12	2.7	32.4	64.9
Group 3 (¾ inch)	8.3	14.0	22.3	7.3	1.21	11.6	41.9	46.5

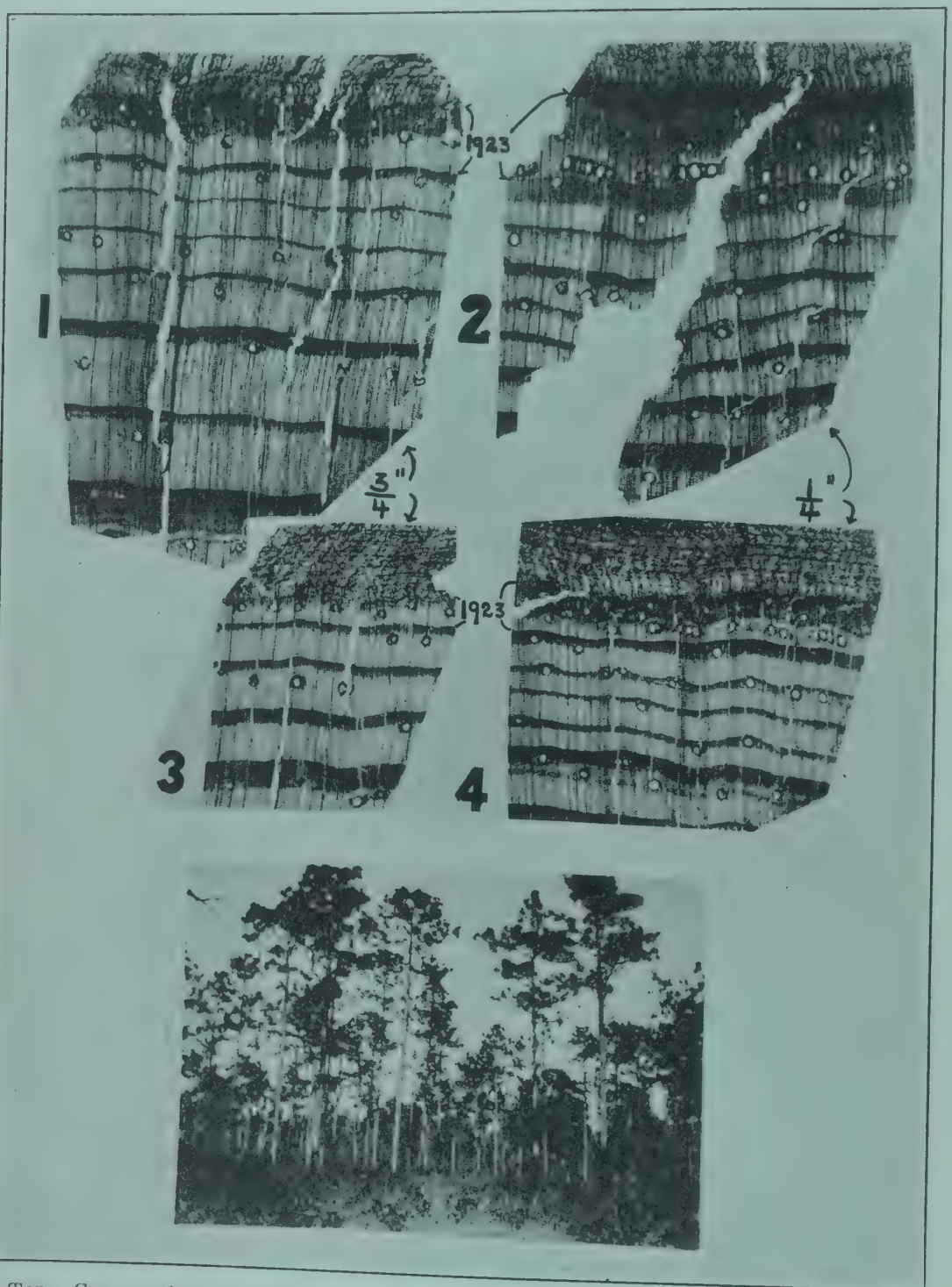
[a] "Unit area" is an arbitrary tangential extent; the diameter of microscopic field by the width of the annual ring observed. Used 32-mm. objective and 15× eyepiece, giving a magnification of about 60 diameters or 3,600 times the actual wood area observed.

The time and amount of summerwood formation appear to be significant factors in judging the effects of given methods of working. Over and over again resiniferous tissue has been found to be produced at the expense of summerwood formation. This is often very striking in the case of trees worked too severely. The production of early and abundant summerwood has been found, so far as the writer's observations extend, to be coincident with conservative working and conditions in which the trees have manifested ability to produce good sustained yields. Hence the fact that the trees in Group 1 had by midseason nearly three times as many summerwood cells as those in Group 3 appears worthy of note.

EXAMINATION AT END OF SEASON.—On November 24, 1923, two weeks

still exceeded by about 50 per cent by those of Group 1. On comparing the width of 1923 rings in each group with the width of those formed in 1922, it is seen (Table IV) that here also the Group 1 trees showed greater vigor of growth than those of Group 3. In Plate 2 are shown typical microscopic sections from chips from these groups. The tendency in Group 3 to produce narrower rings and fewer ducts is plainly evident, as is also the relative width of the 1922 and 1923 rings. At the left (Pl. 2) are shown sections from the most vigorous trees in each group; at the right some from the poorest ones. On the average, Group 1 produced growth rings almost 50 per cent wider than those of Group 3.

Another difference between the trees of Groups 1 and 3 is significant. Certain of the wound-induced resin



Top.—Cross sections of chips. Longest section represents about 1½ inch of wood. Chipping high faces wastes much of the richest and most productive resiniferous tissue before it can yield fully. It cuts away the early-formed, comparatively short resin passages. These are seen in chips 2 and 4 in springwood of the 1923 ring next bark (top), but are lacking in chips 1 and 3.

Bottom.—General view of test area.

passages, particularly those formed in early spring, are comparatively short. By taking off high chips these are cut away before they have had an opportunity to yield the gum which they are capable of producing. With such chipping, potentially valuable wood full of highly organized and productive tissue is cut off and thrown useless on the ground. In Plate 3 it is to be seen that no resin passages are present in the early springwood of the 1923 ring of the specimens from the trees of Group 3. Here the early springwood had probably contained resin passages in June or July, but these, or at least some of them, were short and were probably entirely removed by the time the face reached its final height (21 inches). The cutting away of such resin passages is one of the marked wastes caused by high chipping. To be sure, there still remain many passages which are longer and will continue to produce gum, but a large percentage of available producing power is thus needlessly wasted, despite the fact that conserving it as long as possible presents distinct advantages from every point of view.

Observations have shown that in a given growth ring a considerable reduction in the number of resin passages occurs from 1½ to 3 feet above the early streaks of that year's work. In one instance only half as many resin passages were present in the face at 2 feet above the first streak as were present at 1 foot up. The effect on wood formation of the wound of turpentining may, however, be much further extended vertically. Often the early virgin streaks affect the wood formed for 20 or more feet above, in respect to number of resin passages, width of ring, or amount of summerwood. The greatest number of resin passages, however, have been found in a comparatively limited region of a few feet above the early streaks of a given year.

Low chipping permits the operator to take advantage of this area for the longest possible time. The presence of the many resin passages in the very early springwood of the specimen Group 1 trees represented in Plate 3 is an instance of available productive capacity which may be utilized. The faces on these trees were only 10 inches high. In such trees there is obviously a reserve of highly productive tissue present with which to start the second year of operation. These resin passages will augment the yield until the freshly induced passages of the succeeding year develop and begin yielding at their maximum.

Methods for building up one or more rings of such rich and productive wound tissue before starting regular turpentining are now being tested and present some very interesting possibilities.

DISCUSSION OF RESULTS: NEW WORK

It is common knowledge among practical operators that all that is needed to freshen a streak on a turpentined pine sufficiently to secure a flow of gum is to remove the layer of wood forming the surface of the streak. The dimensions of the layer, however, vary widely in the practical application made by different woodsmen.

After the cut surface of the streak has stood for a few days exposed to the air the wood cells tend to dry and to soak up gum, which they will not do when they are freshly cut and moist. It is then said that the streak has "lightwood" in it. Also, the gum which has flowed out from the resin passages dries and hardens to some extent on the streak surface, owing to evaporation of the volatile spirits of turpentine. At and near the surface of the streak certain of the cells have been injured or killed by the chipping. It is obvious that these destroyed cells and the dried abnormal tissue must be removed.

Undue drying frequently results from chipping too deeply. This practice tends to cause excessive soaking in of gum and the formation of a considerable amount of "lightwood." If such a streak is chipped only ¼ inch high not all the "lightwood" may be removed. This result, however, does not necessarily prove that a higher chip should be cut. Rather it should suggest the possibility that perhaps the too deep chipping has caused interference with the circulation of the watery sap of the tree. It is obvious that enough sapwood should be left behind the face to insure the moisture necessary to keep the living cells healthy and productive. Years of work on the Florida National Forest have shown that with ½-inch-deep chipping healthy conditions are maintained even in trees in which the sapwood is not very wide. Studies of the effect of other depths of chipping are now under way at the Starke station and elsewhere. It is sufficient to say, at this time, that much of the criticism of low chipping, as, for instance, the statement that it "will not keep ahead of the lightwood," or will not remove all the gum-soaked surface of the streak, and that it will radically reduce production, has resulted from observation of trees which were already

chipped so *deeply* that they were drying and soaking unduly. Such trees would obviously not present fair material from which to determine the effect of height of chipping.

Good production has been obtained with the ½-inch-deep and ½-inch-high streak on the by no means superior timber of the Florida National Forest.[12] The question then naturally arises whether or not it is possible to reduce still further the height of the layer of wood removed weekly. Observations indicate that an eighth of an inch, a mere shaving of the surface, will not produce the effects desired; it can not be depended upon to remove the gum-soaked and abnormal surface tissue. In all cases, however, where good average ¼-inch-high chipping of a safe depth has been used, reasonably satisfactory results have been obtained. In view, therefore, of the many indications of good results of conservative chipping in general, and in view of the particular results of this test, it seems safe to work toward chipping lower faces.

The lessened damage to the timber of such turpentining practice is a matter that can not fail to appeal to the owner of the forest and to the sawmill interests. Even the chipper, experience proves, once he has found that it is possible to take off less wood, readily falls into line. To be sure, he is somewhat averse to the longer period during which he must stoop to perform chipping low on the butt of the tree, but he is, on the other hand, spared much longer the hard work of pulling. The expenses of cup raising could be somewhat reduced by cutting lower faces. This is an item worth considering, as is the saving in spirits due to the fact that the gum does not have to go so far to reach the cup. Furthermore, turpentine camps might not need to be moved so frequently. If results continue to demonstrate the fundamental scientific soundness of the principle as well as its numerous practical advantages, the argument in favor of lower faces for commercial production would seem conclusive. At first, perhaps, No. 1 or No. 0 hacks should be used to remove the commercially successful and thoroughly tested ½-inch-high chips, and the streak then gradually reduced to as nearly ¼-inch-high chipping as may be practicable.

For 1924 it is planned to test low chipping at Cogdell, Ga.; Lockhart, Ala.; Holopaw, Opal, Starke, and Camp Pinchot, Fla.; and at several points in Mississippi and Louisiana

SUMMARY OF RESULTS

Evidence obtained from preliminary tests, notably the carefully conducted though comparatively small one here described, and from practical experience indicates that many advantages (reduced scar, less lumber degrade, longer possible operation, higher sustained yield, and conservation of tree energy) may be gained by cutting comparatively low faces (10 inches to 16 inches yearly at most). Such cutting has been satisfactorily accomplished by using a No. "0" hack.

In the test described, three carefully matched groups of 20 trees each were chipped to the same depth (½ inch) and to a height of approximately ¼ inch for Group 1, ½ inch for Group 2, and ¾ inch for Group 3. The Group 1 trees (actual average height of chip 0.28 inch) produced a higher total yield of gum during the first year of the test (1923) than was obtained from the trees of Groups 2 and 3 with higher faces.

The trees with low faces showed earlier and more abundant wood formation (especially summerwood) and a greater amount of gum-yielding tissue (resin passages) than the trees with the higher faces. By midseason (July) they showed nearly twice as many wood cells (including about three times as much summerwood) and about twice as many resin passages formed and ready to function as did the Group 3 trees which were chipped ¾ inch high each week. This would indicate better sustained vitality in the low-chipped trees, and hence would explain the higher producing power which was manifested in their yields.

Preventable waste is caused by high chipping, since it removes more than is necessary of the rich gum-bearing tissue before it has had time to produce the yield of which it is capable. Microscopic study of the number and location in the ring of the resin passages at different heights in the face demonstrated that the early-formed, comparatively short wound ducts are quickly cut away by high chipping. Low chipping permits the longest maximum production in any year, since the face is cut in the region richest in resin passages. Low chipping also makes it possible to hold over a reserve of this tissue to augment

[12] McKee, E. R. NAVAL STORES PRODUCTION ON THE FLORIDA NATIONAL FOREST. Naval Stores Rev. 33 (6): 16–17; (7) 6, 23; (8) 6, 19; (9) 10, 23; (10) 16–17. 1923.

production during a part at least of the following year.

Since extra numbers of resin passages develop as a result of wounding, tests are now under way to determine whether the productivity of low faces can be increased further through stimulating advance resin tissue formation by means of slight wounds (such as inserting the gutter) a year or more in advance of the first regular work.

In the present tests it is significant that although ¼-inch chipping gave a comparatively low yield early in the season it produced during midsummer and fall not only as much as ¾-inch chipping but even more—at the time when severely worked trees frequently show a tendency to dry-face and fail.

The low faces, as compared with the high, showed a considerably increased value of products because of their higher yield. Converting the yield of the 20 test trees in each group to a yield per crop basis, a rough but conservative calculation indicates a difference of $200 in favor of the low faces.

Cutting of low faces (with a streak less than ½ inch high) is under continued test in 1924. On several other small experimental tracts carefully matched slash and longleaf pines of various ages and in varying environments are being worked. It is clearly recognized that the present results obtained from one year's work on a comparatively small number of test trees can only be regarded as indicative. The ¼-inch chipping is now being tried on a commercial scale by practical operators who, for their own information, are comparing the yields from crops or portions of crops (drifts) as the case may be. It is expected that some significant results will be available at the end of the 1924 season.

THE EFFECT OF BACTERIAL NUMBERS ON THE NODULATION OF VIRGINIA SOY BEANS[1]

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Since the discovery that legume nodules are caused by bacteria, many questions have arisen as to the factors which effect the maximum nodulation obtainable. One little-considered factor which affects nodulation is the number of *Bacillus radicicola* organisms present in the substrata. It is but logical to suppose that the greater the number of infecting organisms present the greater will be the number of nodules produced. The proper number of organisms to be used for inoculation is a question since most limed soils favor the reproduction of this organism and, consequently, a scant inoculation will serve to assure the presence of large numbers of infecting organisms. It is maintained by many investigators that after legumes have become nodulated to a certain extent they are immune to additional infection. If this is so, increasing numbers of bacteria should increase nodulation only up to a certain point. The accompanying data show that nodulation will be limited if the number of infecting organisms is limited and that there seems to be an immunity set up in the plant after it has become inoculated to a certain degree.

The nodulation data were obtained from plants grown in washed sand. The sand was fertilized by saturating it with a mixture composed of 0.08 per cent of magnesium sulphate, 0.15 mono basic potassium phosphate, 0.50 per cent calcium carbonate and 0.05 per cent ferrous sulphate dissolved in distilled water. Subsequently, the substrata were maintained 75 per cent saturated by daily waterings with distilled water. The crop was harvested after four weeks' growth. In making the inoculations an emulsion from a one-week-old culture of the organism was standardized by counting with a haemcytometer and diluted to the required degree. The amount of water that a given quantity of seed would absorb was determined and the dilutions were made so that this volume of emulsion would contain the desired number of organisms. Laboratory tests indicated that the fertilizing mixture used would not permit the reproduction of the organism in the absence of an available carbohydrate but that it contained nothing toxic to the bacteria. Therefore it is seen that the substrata used would keep the numbers of infecting organisms practically constant. The plants were grown in tumblers containing one pound of sand. Twenty seeds were planted in each tumbler and the seedlings were thinned out to the ten best. The experiment was conducted in triplicate.

Table I indicates that maximum nodulation may be obtained when the number of infecting organisms applied is between ten and one hundred per seed. Table II shows that maximum nodulation may be obtained when the number of infecting organisms applied is between twenty-five and fifty.

TABLE I.—*Effect upon nodulation of increasing the number of bacteria*

Tumbler No.	Number of organisms applied per seed	Plants inoculated	Number of nodules per 10 plants
		Per cent	
1A	0	0	0
1B	0	0	0
2C	0	0	0
2A	1	0	0
2B	1	0	0
3C	1	0	0
1A	10	40	12
3B	10	80	24
3C	10	70	25
4A	100	100	63
4B	100	100	73
4C	100	100	59
5A	1,000	100	71
5B	1,000	100	65
5C	1,000	100	80
6A	10,000	100	72
6B	10,000	100	78
6C	10,000	100	84

[1] Received for publication June 11, 1924. Paper No. 173 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology. Part of a thesis submitted to the faculty of Rutgers College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The author wishes to express his appreciation to Dr. J. G. Lipman for helpful suggestions in planning this work.

TABLE II.—*Effect upon nodulation of increasing the number of bacteria*

Tumbler No.	Number of organisms applied per seed	Plants inoculated	Number of nodules per 10 plants
		Per cent	
1A	0	0	0
1B	0	0	0
1C	0	0	0
2A	1	0	0
2B	1	0	0
2C	1	0	0
3A	10	1	3
3B	10	1	5
3C	10	1	4
4A	25	100	48
4B	25	100	62
4C	25	100	51
5A	50	100	60
5B	50	100	68
5C	50	100	71
6A	100	100	59
6B	100	100	66
6C	100	100	73
7A	500	100	65
7B	500	100	78
7C	500	100	63

Both tables show that a high number of infecting organisms will not give a better inoculation than a moderate number. Theoretically, it seems that infection and nodulation are partly a hit-or-miss affair. When a limited number of nodular organisms are present the chances of contact between host and bacteria are naturally not so great as when a large number of organisms are present. Therefore the greater the number of infecting organisms the greater should be the number of nodules produced. This ratio between bacterial numbers and nodulation holds only when the number of organisms is very limited and approaches infinity as the number of bacteria is increased. It is therefore apparent that there must be a maximum infection obtainable and that after this maximum is reached the plants become immune to additional infection.

The conclusions to be drawn are:

1. When the number of nodular organisms is limited there is a distinct relation between the number of organisms present and the number of nodules formed.

2. There is a rather definite minimum number of nodular organisms required to produce maximum infection.

3. After a certain degree of infection is reached the host is immune to additional infection.

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CLASSIFICATION OF SCALE INSECTS OF THE SUBFAMILY ORTHEZIINAE [1]

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INTRODUCTION

The subfamily Ortheziinae forms a well defined group within the family Coccidae. The group is of interest not only on account of the wide distribution and fully recognized injuriousness of certain of its members, but because of the characteristic and even, for Coccidae, beautiful superficial appearance produced by the usually sharply defined, platelike tufts of waxy secretion, and the rather unusual morphological modifications of the body structure. The group has a further interest in this country in that, so far as its species are known, it is predominantly American, presumably both in origin and in development.

Although only one of the species at present found within the United States, the so-called greenhouse Orthezia, has attracted attention as a scale pest of major importance, the presence of a relatively large number of species in the Southwestern States and the potentially wide host range indicated for the subfamily by the known host records, combined with the marked increase in agricultural development in this southwestern region in recent years, at least suggests the possibility that the members of the subfamily may take on a new economic importance if the proper conditions for the increase and spread of some of the species found there are created through the continued expansion of agricultural activities in this region.

ILLUSTRATIONS

In making use of the illustrations certain factors should be taken into consideration. Practically all of them, both the photographs and the drawings, have been made from dried specimens, most of which have been preserved for years. It is difficult, with many of the species, to obtain perfect specimens even in life, and the condition after years of storage is usually such that few, if any, of the specimens shown in the photographs are perfect. The same and other conditions have affected the specimens which have been mounted, with the result that hardly any of the drawings of the insects as a whole are to be regarded as representing the structures figured with entire correctness and in precisely coordinated relation to each other. Thus, while the dorsal and ventral spine bands in the abdominal region are believed to be depicted in fairly accurate fashion, it has frequently been quite impossible, from the material at hand, to make absolutely certain of the size, shape,

[1] Received for publication September 4, 1924; issued April, 1925.

[2] Only the availability of the large collection of Coccidae built up during past years through the work of the Bureau of Entomology and through gifts of specimens to it and to the United States National Museum by various individuals has made this paper possible. Since beginning the study the writer has become indebted to Prof. T. D. A. Cockerell, Prof. E. O. Essig, and Prof. G. F. Ferris, in the United States, to Mr. Adolph Hempel, of Sao Paulo, Brazil, and to Dr. F. Silvestri of Portici, Italy, for the loan or gift of valuable specimens which have greatly aided the work on the group. The writer has been able to examine either type or apparently authentic material of 34 of the 40 species accepted as valid in this paper, including all but 2 of the species of Orthezia.

Most of the figures included in this paper to illustrate the general appearance of the body and particularly the arrangement of spine bands and clusters in the species discussed have been prepared, under the direction of the writer, by Miss Sarah Hoke, while Miss M. E. Stehle (Mrs. J. C. Hamlin), Miss A. I. Shoemaker, and Miss Leola J. Kruger have each contributed a few. Nearly all of the photographic illustrations have been prepared by J. G. Pratt.

Owing primarily to space limitations it has been found necessary to reduce the bibliographical citations under each species to the few required to show the original publication of the description and responsibility for any accepted synonymy. Many references will be found in the Fernald "Catalogue of the Coccidae of the World," a publication almost always accessible to working entomologists, and many others may be obtained by consulting the papers of the present-day active workers on the Coccidae.

and position of the anterior bands and spine clusters, particularly in the ventral thoracic and head regions, and the arrangement of these as figured will probably prove to be only approximately correct when perfect specimens of each species are available for study. Again, owing to the viccissitudes incurred during the mounting of specimens often imperfect, the body has frequently become distorted in such a fashion as to make its true outline very uncertain and, in consequence, the body outline of any species, as shown in its figure, should not be regarded as necessarily correct, nor should any variation in this among species be regarded as of taxonomic importance. The figures showing dorsal and ventral halves of the body are also incomplete in that they make no attempt to show and locate all of the setae and disk pores present in the derm of the insect, usually showing only those that appear to have some obvious or possible importance in relation to their classification. In spite of any such deficiencies, it is believed that these figures, if used in connection with the text, will greatly facilitate the recognition of the different species discussed in this paper.

In the detail drawings of the spines from certain parts of the body of each species, an attempt has been made to show the average condition for each. Usually, however, since nearly all of these spines have not only a curved longitudinal axis, but vary from circular to oval in cross sections at different points, the angle at which any spine lies under the microscope has a marked influence on its profile and should be given consideration when the spines are under examination.

Most, but not all, of the photographic illustrations show the insects enlarged approximately seven and one-half times. The text should be consulted when accurate information as to the size of a species is desired. The enlargement in the drawings varies and is indicated for each.

GEOGRAPHICAL DISTRIBUTION

The original natural distribution of the species of this subfamily, necessarily inferred in the case of some of the more widely distributed forms such as *insignis*, shows two rather striking characteristics which deserve mention. Thus, so far as records are available, there are no truly indigenous species in the Ethiopian, Oriental, and Australian regions, and the known species are predominantly Nearctic and Neotropical in distribution and only secondarily Palaearctic.

The 40 members of the subfamily definitely recognized at present are given in the list below and their general natural distribution throughout the world is indicated on the accompanying maps (figs. 1 and 2).

ORTHEZIA BOSC D'ANTIC

Orthezia s. str.
- *annae* Cockerell.
- *arenariae* Vayssière.
- *artemisiae* Cockerell.
- *balloui*, new species.
- *boliviana*, new species.
- *caudata* Ferris.
- *cheilanthi* Tinsley.
- *galapagoensis* Kuwana.
- *garryae* Cockerell.
- *graminis* Tinsley.
- *grandis* Hempel.
- *insignis* Douglas.
- *lasiorum* Cockerell.
- *longipes* Hempel.
- *mexicana*, new species.
- *minor*, new species.
- *monticola* Cockerell.
- *nigrocincta* Cockerell.
- *nuda* Ferris.
- *olivacea* Cockerell.
- *praelonga* Douglas.
- *pseudograminis*, new species.
- *solidaginis* Sanders.
- *sonorensis* Cockerell.
- *tillandsiae*, new species.
- *ultima* Cockerell.
- *urticae* (Linnaeus).
- *varipes* Leonardi.
- *yashushii* Kuwana.

Arctorthezia Cockerell.
- *cataphracta* (Shaw).
- *occidentalis* Douglas.

NEWSTEADIA GREEN

- *americana*, new species.
- *floccosa* (De Geer).
- *tristani* (Silvestri).

MIXORTHEZIA, NEW GENUS

- *cubana*, new species.

ORTHEZIOLA SULC

- *fodiens* Giard.
- *vejdovskyi* Sulc.

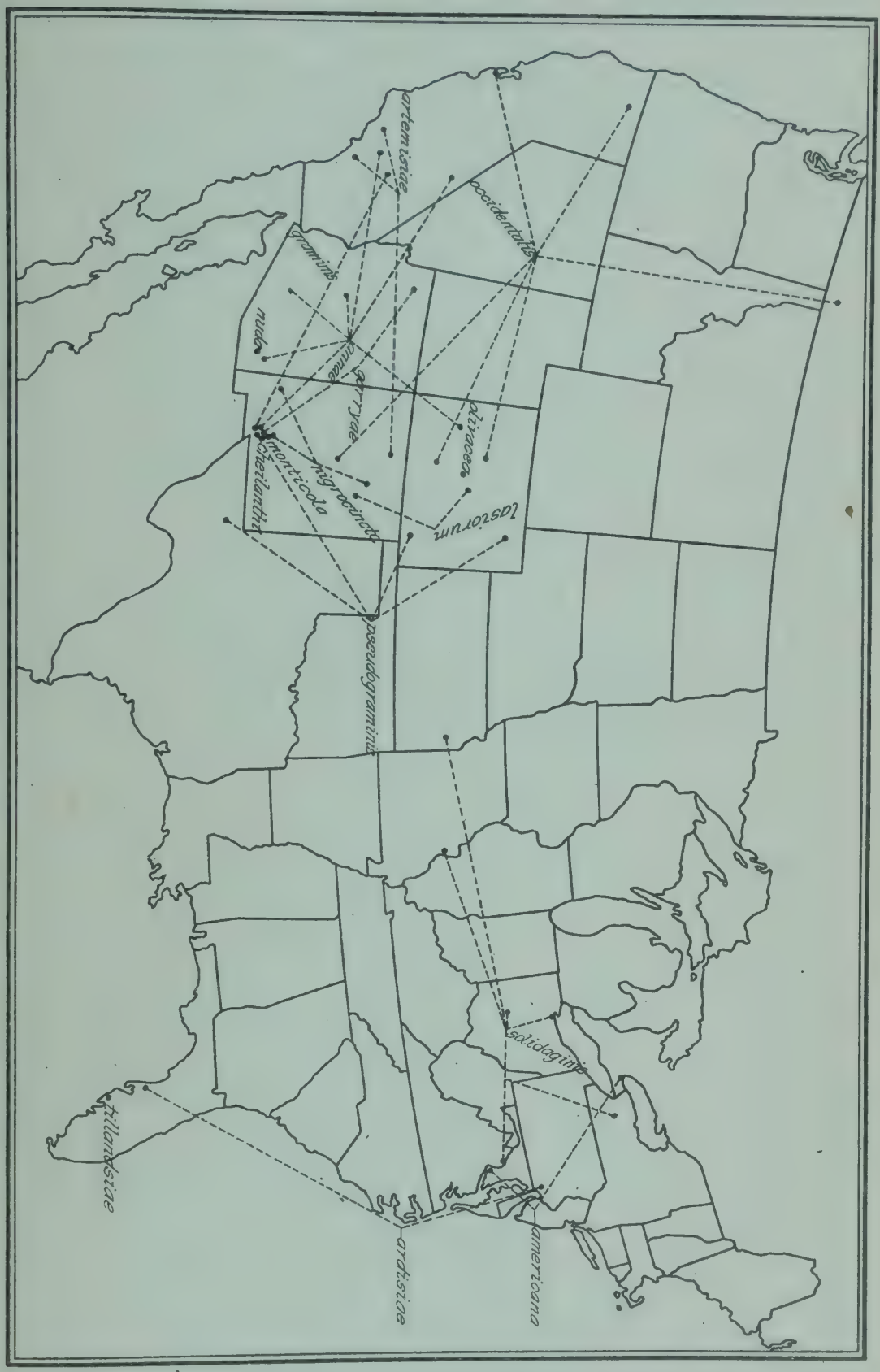

FIG. 1.—Map showing the distribution of the species of Ortheziinae known to be present in the United States

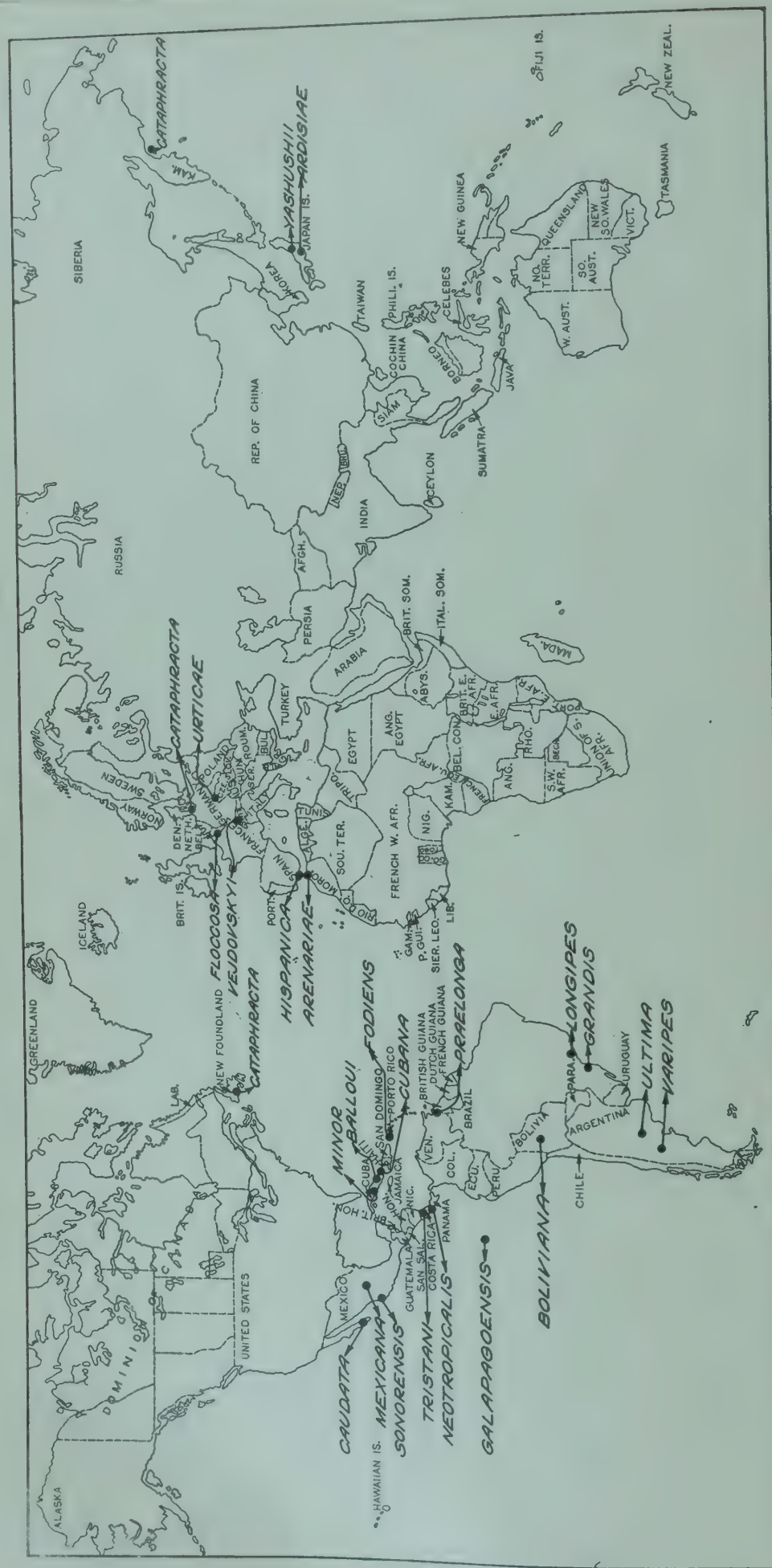

FIG. 2.—Map showing the approximate natural distribution of the species of Orthezиinae, excluding those present only in the United States

NIPPONORTHEZIA KUWANA

ardisiae Kuwana.
hispanica (Silvestri).
neotropicalis (Silvestri).

One species, *Orthezia cataphracta*, is known through definite records to be Holarctic in its distribution. A second species, *Nipponorthezia ardisiae*, has been found both in Japan and in the Eastern United States and, as its origin is uncertain, although the present evidence favors the assumption that it is probably native to the United States and was introduced into Japan, its occurrence in both localities has been indicated. The distribution of each of the other species is shown by the use of its name a single time only, this, in each case, being placed as nearly as possible at the location indicated by the known collection records, or as nearly as possible at the center of its known distribution, where the species is already widespread. In addition to the one Holarctic species and the Japanese species mentioned, 17 species are to be credited to the Nearctic region, nearly all of which occur in the southwestern portion of the United States or in northern Mexico. One other species, *tillandsiae*, from Florida, should possibly be included here, but in its general appearance, its morphological characters, and its host relations it seems to belong more properly in the upper, insular, section of the Neotropical region with *balloui* and *minor*. Including these 3 species, 14 species are to be credited to the whole Neotropical region, making a total of 33 species known to occur on the continents of North and South America and adjacent islands. This leaves, aside from the Holarctic species already mentioned, only 7 species represented in the Palaearctic region, one of which is known from a single locality in Spain, one from a single locality in Morocco, and two from Japan, while the other three are known from sufficiently separated areas to indicate their wide distribution through at least the western portion of the Palaearctic region. The summary of the distribution of the species by regions therefore stands as follows: Palaearctic, 7 (including 2 also Nearctic); Nearctic, 19 (including 2 also Palaearctic); Neotropical, 14; and Insular, 1 (*galapagoensis*).

The actual known distribution of a few of the more important species is shown with precision in small outline maps accompanying their descriptions.

HOST RELATIONSHIPS

The records of the infestation of various plant hosts by the 40 species of this subfamily come from such a wide range of families in the plant kingdom that about the only generalization possible for the subfamily is that some one of its members might, under proper conditions, attack almost any of the higher growing plants. Even Coniferae, although not included among the hosts of any of the described species, have been reported as harboring a species of Orthezia. A summary of the records for the various species indicates, however, a predominance of certain plant families as hosts for the members of the subfamily. Conclusions regarding such predominance are, of course, accurate only in so far as our present knowledge extends, and can not be understood to indicate definitely the host limitations of any one of the species, or even necessarily its preferred host plants, since, in the case of the more important and more widely distributed species, such as *insignis*, *praelonga*, and *urticae*, the range of host families is rather extended and appears to depend almost as much upon the availability of the host to the insect as upon any other factor.

Of the known species of Ortheziinae, 8 appear to have members of the Compositae as the only or predominant hosts; 4 have species of the Gramineae as the hosts; 2 have members of the Rubiaceae as the only known hosts; 2 more have genera in the Chenopodiaceae as their only hosts; and members of the Bromeliaceae, Euphorbiaceae, Fagaceae, Melanostomaceae, Myrsinaceae, and Polypodiaceae are infested by one coccid species to each family. In the case of 6 of the described species, including the two important pests *insignis* and *praelonga*, it is not possible to cite any one family as including the most acceptable hosts, although, as recently pointed out by E. E. Green for *insignis*, the tendency in its case appears to be to prefer as hosts members of the families Acanthaceae, Compositae, Verbenaceae, and possibly, in addition, Solanaceae, and, in the case of *praelonga*, members of the Euphorbiaceae. The plant hosts of the remaining species are unknown, owing either to failure or inability to make a record at the time of collection, or to the finding of the species in ants' nests not directly associated with any host plant.

The discussion of the classification and of the detailed distribution and host relationships of the subfamily and its included species follows.

SUBFAMILY ORTHEZIINAE

The members of this subfamily may be distinguished from other Coccidae by the following combination of characters:

Adult female with fully developed abdominal spiracles; anal ring with a pore band present, more or less distinctly divided into right and left halves and these into inner and outer sections and bearing 6 anal ring setae; antenna with a single stout terminal spine or seta; each leg with at least the trochanter and femur fused into a single part; beak 1- to 2-segmented; derm pores of the quadrilocular disk type, rarely with some multilocular or clustered pores; body with a few slender setae and numerous rather stout spines, the latter grouped in definite bands and clusters; external secretionary covering, in so far as developed, made up of definite tufts or plates of waxy secretion forming a distinct pattern varying with the species; mature insect with a prominent posterior ovisac, often much longer than the insect itself, this secreted by a well developed ventral ovisac band; active throughout the whole adult stage.

Larva similar to adult female in the possession of the abdominal spiracles, anal ring with pores and setae, and spines in more or less definite clusters; antennae 3- to 6-segmented.

Distinguishing characteristics of adult males not sufficiently known to permit their differentiation from the males of other groups having compound eyes and abdominal spiracles. An incomplete diagnosis of those of Orthezia may be found under the discussion of that genus.

In the possession of the abdominal spiracles, presumably in all stages, and in the possession of the compound eyes, accompanied by ocelli, in the adult males, the members of this subfamily show a rather close relationship with the group of Coccidae at present placed under the subfamilies Monophlebinae and Margarodinae, and it appears that the subfamily Ortheziinae is more closely related to this group than to any other within the family. The species of Ortheziinae depart distinctly from the Monophlebinae and Margarodinae in having, in both adult female and larva, an anal ring with distinctly developed pore bands and anal ring setae. The fusion of trochanter and femur, although characteristic for the subfamily and accompanied by a comparable fusion of the tibia and tarsus in all of the genera except Orthezia, is not distinctive, as a similar coalescence of parts of the legs occurs occasionally in some other subfamilies, although in such cases this coalescence is usually accompanied by a marked reduction in the size and functioning capacity of the legs.

The known genera of this subfamily may be distinguished by the following key:

KEY TO GENERA OF ORTHEZIINAE

a. Tibio-tarsal articulation distinctly developed; antennae usually normally 8-, rarely 7-segmented, if the latter, the basal segment not very large and prominent, and the abdomen with 7 or 8 pairs of spiracles ..... ORTHEZIA Bosc.

aa. Tibio-tarsal articulation wanting, or at most suggested by a transverse line, these two parts of the leg rigidly fused; antennae usually normally with fewer than 7 segments, or, if with this number, the basal segment very large and prominent.

b. Antennae 6- or 7-segmented; with 5 pairs of abdominal spiracles, these present on the anterior segments ..... NEWSTEADIA Green.

bb. Antennae at most 4-segmented.

c. With 8 pairs of abdominal spiracles as in Orthezia; antennae 4-segmented ..... MIXORTHEZIA, new genus.

cc. With fewer than 8 pairs of abdominal spiracles; antennae actually 3-segmented.

d. Antennae apparently 4-segmented; eyestalk and pseudo-basal antennal segment fused; beak 1-segmented; anal ring with 6 subequal setae; abdominal spiracles uncertain ..... ORTHEZIOLA Sulc.

dd. Antennae 3-segmented; eyestalk and basal antennal segment distinctly separate; beak distinctly 2-segmented; anal ring with the intermediate pair of setae much smaller; with 6 pairs of abdominal spiracles ..... NIPPONORTHEZIA Kuwana.

GENUS ORTHEZIA BOSC D'ANTIC

Reference.—*Orthezia* Bosc, 1784, Jour. Phys. 24: 173.

Synonyms.—*Dorthezia* Bosc, *Cionops* Leach, *Cyphoma* Gistel (see Fernald, 1903, Cat. Cocc. World, p. 33), *Polyocellaria* Imhof, 1900, Biol. Centbl. 20: 527; *Douglasia* MacGil-

livray (preoccupied); *Douglariella* MacGillivray (see Laing, 1922, Ent. Mo. Mag. 58: 254–255).

The primary distinguishing characters separating this genus from the other genera of the Ortheziinae have been indicated in the key already given. The genus may be diagnosed as follows:

ADULT FEMALE.—External covering, so far as developed, made up of definite and usually sharply segregated tufts of waxy secretion, these often quite long or high, more or less completely covering the body and arranged in distinct marginal and dorsal rows, the dorsal rows, when fully developed, 10 in number on each half of the body, the marginal, including that on the head and a transverse median plate at the posterior end of the body, 10 or 11 in number on each side of the body; body more or less distinctly oval, often broadly rounded posteriorly and somewhat tapering anteriorly; derm entirely membranous, or with small, dark-colored chitinized plates dorsally in varying position; antennae rather heavily chitinized, 7 to 8 segmented, the basal one or two segments not conspicuously enlarged; eyestalk varying from flat conical or tuberculate to elongate, thumblike; legs long, joint between trochanter and femur obsolete, the parts bearing a number of setae, these usually spinelike and set in definite rows, tarsal claw long, usually with denticles on the inner face, claw digitules spinelike; beak 1-segmented, with a more or less distinct suggestion of a joint near base in many species; the usual 2 pairs of thoracic spiracles present, these distinctly larger than the abdominal spiracles, the latter more or less elongate tubular, with a collar at opening, placed along margin or just within it dorsally, occurring certainly in 7 or 8 pairs, and apparently sometimes in 4 pairs, the posterior 2 on each side always placed quite close together and diagonally behind the anal ring; derm pores of the quadrilocular disk type only, these varying in size and density of chitinization, distributed over the body both dorsally and ventrally, usually in single rows between the clusters and bands of spines and in transverse rows or bands in the ventral abdominal region, rarely individually abnormal in structure, as trilocular or quinquelocular; body setae slender, set in distinct, flat, basal collars, occurring sparingly over most of the dorsum and venter but often in a distinctly developed cluster anterior to the genital opening; body spines grouped in definite clusters and bands, these, when fully developed, in 10 or 11 clusters along the margin and in 10 bands dorsally on each half of the body, in a distinct and usually broad ovisac band around the ventral margin of the abdomen, and usually in transverse rows or bands across the area inclosed by this band; dorsal bands and rarely the marginal clusters in some species more or less reduced in area, leaving exposed bare dermal areas, these usually taking on the form of longitudinal submarginal stripes, one on each side of the body; spine clusters and bands serving as bases for the tufts of secretion in the perfect insect, and the arrangement of the secretionary tufts in consequence corresponding rather closely to that of the spine clusters; ovisac band, in addition to the spines, often with disk pores along outer and inner margins and frequently through more or less of the entire width of the band; anal ring flat, more or less distinctly oval, bearing 6 relatively long, tapering setae, and, on each half, a distinct, but not always completely developed band of pores, this usually more or less evidently divided into inner and outer sections, and rarely with a small, more or less triangular, chitinized wing projecting laterad from the middle of each half of the band.

LARVA.—Body more or less distinctly oval, antennae 6- or very rarely 5-segmented; eyestalk more or less conical, in each species corresponding vaguely to the shape of this structure in the adult female; legs about as in adult female; thoracic spiracles much larger than abdominal; abdominal spiracles very difficult to locate, but, so far as placed, similar in number and shape to those of adult female; derm pores of the quadrilocular disk type; derm setae occurring occasionally; spines arranged in dorsal and marginal clusters and ventral rows, agreeing very vaguely with the condition in the adult female of the same species; anal ring with the usual pores and setae.

ADULT MALE.—Elongate, slender, abdomen with sides parallel or nearly so; thoracic lobes chitinized, remainder of body membranous with exceptions noted below; antennae 9-segmented, the 2 basal segments relatively short and stout, remainder very long, cylindrical, apical terminating in a spine as in female, hairs on outer segments rather numerous, fairly stout, of moderate length, more or less distinctly recumbent, arranged in indefinite spirals; head triangular in out-

line anteriorly; compound eyes large, each accompanied behind by a protruding ocellus; legs long and slender, the parts cylindrical, bearing numerous spines or setaelike hairs, the tarsal claws each with 2 digitules and usually 1 or 2 denticles near apex; wings rather long and slender, usually showing more or less whitish bloom on the surface, costal margin thickened, with 1 subbasal diagonal thickening or vein running nearly to margin, a more or less distinct clear line somewhat beyond and paralleling this, and rarely (*praelonga*) a second diagonal thickening intermediate in position between costal and basal diagonal veins, less distinctly developed than either and incomplete basally; abdomen terminating in a bivalved penis sheath, this curving back and down, with the tips of the valves closely appressed and forming a rounded conical apex, and on a single abdominal segment before this sheath with a transverse row of long tubular ducts, these producing the tuft of glassy threads to be seen in the living insect.

The preceding diagnosis of the adult male stage has been based on males of only four species and in consequence may not be entirely accurate for all the members of the genus. Neither thoracic nor abdominal spiracles were to be observed in any of the specimens examined, although almost certainly present. No definite group or specific characters were observed in the few species examined, and their existence seems somewhat doubtful, although a detailed study of the more minute derm structures might possibly result in the discovery of such characters.

One subgenus, which has also been regarded as a genus by some, has been erected within the genus Orthezia and appears, on the basis of the studies detailed here, to be valid. It may be separated from typical Orthezia by the key following.

KEY TO SUBGENERA OF ORTHEZIA

a. Without 3 triangular clusters of spines on the anterior portion of the dorsum along the median line, and the corresponding triangular plates of secretion also wanting; when fully developed, with 11 marginal and 10 dorsal pairs of clusters or bands of spines, and with a corresponding number of plates or tufts of secretion; eyestalk sometimes strongly asymmetrically conical, but never thumblike; tarsal claw with denticles. ORTHEZIA s. str.

aa. With 3 more or less distinct triangular clusters of spines on the anterior portion of the dorsum along the median line, and with 3 distinct triangular plates of secretion corresponding to these; clusters of spines fully developed, forming only 10 pairs of marginal clusters instead of 11, the secretion of the perfect insect corresponding; eyestalk strongly produced, thumblike; tarsal claw without denticles ARCTORTHEZIA Cockerell.

SUBGENUS ORTHEZIA S. STR.

The characters of the subgenus Orthezia s. str. have been sufficiently indicated in the generic diagnosis of the various stages and in the subgeneric key given above.

At least one of the members of this subgenus, owing quite evidently to artificial dissemination, is at present world-wide in its distribution, although, as has already been indicated, the subgenus appears to be American in origin and development, the majority of the described species coming from the Western and Southwestern States and nearly all of the remainder from tropical or Neotropical America.

In the matter of host relationships the members of the subgenus, largely because of the diverse tastes of a few species, show so wide and varied a range that no precise connection between the subgenus as a unit and the hosts of its members is evident.

Among the members of this subgenus are present some fairly evident groups of species, and, in addition, some species which appear to stand alone. As these suggested groups can not be successfully placed in any linear arrangement and are not indicated in the key to the species which follows, or in the arrangement of the specific descriptions, it seems desirable to outline them very briefly, with the definite understanding that the order of arrangement as given in the following tabulation is not intended to show any proper phylogenetic or other sequence.

I. *insignis* Dougl. Characterized primarily by the complete absence of transverse rows or bands of spines in the ventral abdominal region and, secondarily, by the very marked reduction of the dorsal spine bands and corresponding secretion.

II. *graminis* Tins., *monticola* Ckll., and *pseudograminis*, n. sp. Characterized primarily by the occurrence of more or less distinct bare derm stripe between the dorsal and marginal clusters of spines and corresponding tufts of secretion, in combination with the occurrence of 7 pairs of abdominal spiracles, the absence of any chitinized dermal areas, the presence of a flattened conical eyestalk, and an anal ring with the inner margin of the pore bands not strongly angulate.

III. *artemisiae* Ckll., *galapagoensis* Kuw., *garryae* Ckll., *longipes* Hemp., *praelonga* Dougl., *ultima* Ckll., *varipes* Leon. Characterized primarily by the presence of an anterior, dorsal, median, chitinized derm plate extending posteriorly between the anterior marginal spine clusters, in combination with the presence of 7 pairs of abdominal spiracles, and usually a strongly conical eyestalk.

IV. *mexicana*, n. sp. Characterized primarily by the substitution, in the ventral abdominal area, of transverse rows of truncate conical tubercles for the usual transverse rows of spines, and secondarily by the absence of chitinized derm plates, the presence of 7 pairs of abdominal spiracles, complete dorsal spine bands and secretion, and numerous pores through the outer fourth of the ovisac band, but none along or through the inner margin.

V. *caudata* Ferris. Characterized primarily by the presence, in the dorsal spine bands and to some extent in the marginal clusters, of a few spines very much larger, more heavily chitinized, and much more conspicuous than the average, and secondarily by the presence of a median dorsal anterior chitinized plate as in the *garryae* group, but in combination with 8 pairs of abdominal spiracles instead of 7.

VI. *balloui*, n. sp., *minor*, n. sp., *tillandsiae*, n. sp. Characterized primarily by the apparent presence of only 4 pairs of abdominal spiracles, and secondarily, in two of the species only, by the presence of conspicuous dorsal abdominal plates in connection with the dorsal spine bands, by small size, and by subtropical distribution.

VII. *annae* Ckll., *nuda* Ferris, *nigrocincta* Ckll., *sonorensis* Ckll. Characterized primarily by the more or less marked reduction in size and shape of the spines in the collar surrounding the opening of each thoracic spiracle, and by a more or less incompletely developed anal ring, with the pore bands of this hardly angulate within and often with a more or less distinct lateral chitinized wing, in connection with the presence of 8 pairs of abdominal spiracles, and, except in *nigrocincta*, the absence of any dermal chitinization.

The species *nuda* has been somewhat doubtfully included in this group, as its structures diverge more or less distinctly from the primary characteristics indicated, and in addition it lacks some, at least, of the marginal and most of the dorsal spine clusters and secretionary tufts, while the remainder are much reduced in size.

VIII. *arenariae* Vays., *boliviana*, n. sp., *cheilanthi* Tins., *grandis* Hemp., *lasiorum* Ckll., *olivacea* Ckll., *solidaginis* Sanders, *urticae* (L.), *yashushii* Kuw. Characterized primarily by the presence of complete dorsal spine bands and secretion, 8 pairs of abdominal spiracles, 5 transverse rows of spines in the ventral abdominal region, and a usually distinctly conical eyestalk, in combination with the absence of any dermal chitinization.

Some of the species, as *cheilanthi* Tins., with slightly clavate derm spines, and *lasiorum* Ckll. and *olivacea* Ckll., with normally 7-segmented antennae, tend to diverge from the characteristic condition in this group as represented by the species *urticae* (L.).

With the exceptions indicated in the footnote, the known species of the subgenus Orthezia have been included in the following key:

KEY TO SPECIES OF THE SUBGENUS ORTHEZIA [3]

a. Ventral abdominal area, inclosed by ovisac band, without any transverse rows of spines; dorsal secretion confined to small tufts forming two complete, but narrow, longitudinal, submedian bands and exposing a wide expanse of bare derm between dorsal and marginal secretion of each side---------- *insignis* Douglas.

aa. Ventral abdominal area with at least some spines in transverse rows, or, if without spines, with numerous small truncate conical tubercles in similar transverse rows, the dorsum in the latter case completely covered by secretion and with complete spine bands.

b. Dorsal spines confined to 5 small pairs of clusters along the median line on the posterior abdominal segments, secretion similarly limited, most of the dorsum completely exposed; marginal spine clusters and corresponding secretion obsolete anteriorly, ventral abdominal bands of spines poorly developed, each a single row of scattered spines---------- *nuda* Ferris.

bb. Dorsal spines, at the least, forming two complete median longitudinal rows as in *insignis*, usually forming more or less complete, wide, transverse bands; marginal spine cluster pairs complete, 11 in number; ventral abdominal bands of spines usually distinctly developed, and even broad; dorsal and marginal secretion corresponding to the spine arrangement.

c. Transverse rows of spines in ventral abdominal area replaced by similar rows of small truncate conical tubercles, each heavily chitinized, but with nearly membranous cap; ovisac band with numerous disk pores through the outer fourth, but without these along or through the inner margin; dorsal spine bands and secretion very well developed; 7 pairs of abdominal spiracles---- *mexicana*, new species.

cc. Transverse bands of spines in ventral abdominal area unmodified; ovisac band varying according to the species; dorsal spines and secretion varying.

d. Some of the spines, in patches among the dorsal spine bands, conspicuously larger and more heavily chitinized than the average; dorsal spine bands and secretion complete; 8 pairs of abdominal spiracles and an anterior, median, elongate, dorsal chitinized derm patch-------- *caudata* Ferris.

dd. All dorsal and marginal cluster spines approximately uniform in size, shape, and chitinization; other characters varying.

e. Spines making up spine collar surrounding opening of each thoracic spiracle very short conical, differing conspicuously in shape from remaining body spines; 8 pairs of abdominal spiracles; anal ring not sharply angulate within anteriorly and posteriorly, often with a distinctly developed chitinized wing on each side; inner spines of the 2 posterior dorsal clusters more or less distinctly reduced in size as compared with the remaining spines.

f. Dorsal spine bands on thorax and anterior abdominal segments, while transverse, terminated far from the corresponding marginal clusters, leaving a wide, bare, longitudinal derm band between dorsal and marginal spine clusters; secretion corresponding, leaving that portion of the derm exposed in perfect specimens; lateral wings of anal ring not developed or only slightly so; ovisac band inclosing only 3 transverse spine bands---------- *nigrocincta* Cockerell.

ff. Dorsal spine bands continuous almost to corresponding marginal bands, although with the abdominal bands narrowed laterally, the dorsal surface, in perfect specimens, not, or only very slightly exposed; lateral wings of anal ring well developed, very evident; pore bands of anal ring incompletely developed; ovisac band inclosing 5 transverse bands of spines.

g. Larval antennae 6-segmented; inner spines of the 2 clusters just before anal ring conspicuously smaller than the remainder in the larva---------- *annae* Cockerell.

gg. Larval antennae 5-segmented, the third very long and more or less constricted medially; none of the spines in the clusters just before anal ring conspicuously reduced in size in larva---------- *sonorensis* Cockerell.[4]

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[3] The species *americana* (Walk.), *arenariae* Vays., and *yashushii* Kuw. have been omitted from this key, as no specimens of any of the three are available for comparative study. The status of *americana* will probably never be determined with certainty, but the other two species, from the descriptions, are evidently very closely related to *urticae* (L.).

[4] No clear-cut and distinct morphological differences that will definitely separate the adult females of these two species, *annae* and *sonorensis*, have as yet been isolated; some comparative and apparent differences are indicated in the descriptions and figures of the two species.

ee. Spine collar around opening of each thoracic spiracle, if developed, made up of spines similar in shape to and, at most, only a little smaller than the remaining body spines; inner spines of those in the two posterior dorsal clusters not obviously reduced in size in comparison with the remainder; other characters varying.

h. Outer halves of the dorsal spine bands of the 4 intermediate abdominal segments displaced on each side of the body by 4 trapezoidal, heavily chitinized plates; size small, length as mounted little more than 1 mm.; apparently with only 4 pairs of abdominal spiracles ..... *balloui*, new species.

hh. Body chitinization, if developed, not as described above, never interrupting the abdominal spine bands; other characters varying.

i. With 3 more or less distinctly developed, chitinized, oval plates interposed between the outer ends of the intermediate dorsal abdominal spine bands on each side; length as mounted about 1.3 mm.; apparently with only 4 pairs of abdominal spiracles ..... *tillandsiae*, new species.

ii. Body chitinization, if developed, not as described above; other characters varying.

j. With a more or less elongate or oval, dorsal, chitinized derm plate extending caudad from the anterior body margin for about the depth of the anterior marginal spine band, this plate frequently obscured by the mouth parts in mounted specimens; with 7 pairs of abdominal spiracles.

k. Ovisac band inclosing 4 definite transverse spine bands, these all relatively narrow.

l. Disk pores widely scattered through the inner two-thirds of the anterior median section of the ovisac band, as well as in a band along its inner margin; no visible row of such pores along the outer (anterior) margin of this section of the band; chitinized thickening stout oval ..... *varipes* Leonardi.

ll. Disk pores, at most, present only through the inner one-third of the anterior median section of the ovisac band; with a row or band of disk pores along the outer (anterior) margin of this portion.

m. Anterior median section of ovisac band relatively narrow, as little as 8 to 10 spines wide; with a single to irregularly double row of pores along outer (anterior) margin, and a band about 3 wide along and through the inner (posterior) margin; antennae slender, length about 0.9 mm.; dorsal spine bands incomplete; known only from the Galapagos Islands ..... *galapagoensis* Kuwana.

mm. Anterior median section of ovisac band relatively much wider, at least 14 spines wide, other characters varying.

n. Antennae normally relatively short and with broad segments, about 0.6 mm. long; dorsal spine bands, particularly on the abdominal segments, incomplete, tapering at outer ends; anterior median section of ovisac band with a single row of disk pores along outer (anterior) margin; dorsal chitinized thickening oval ..... *ultima* Cockerell.

nn. Antennae normally long and more slender, exceeding 1 mm. in length; dorsal spine bands continuous to the marginal, outer ends somewhat expanded, the individual bands on the abdomen quite narrow; anterior median section of ovisac band with a double to quadruple row of disk pores along outer (anterior) margin.

o. Anterior median section of ovisac band with only a single row of disk pores along inner (posterior) margin, but with a triple to quadruple band along outer (anterior) margin; eyestalk asymmetrically flattened conical; chitinized thickening oval ..... *garryae* Cockerell.

oo. Anterior median section of ovisac band with a band of disk pores 3 or 4 wide along and through the inner (posterior) margin, and with a double to triple band along the outer (anterior) margin; eyestalk at least strongly rounded conical; chitinized thickening at most elongate triangular.

p. Eyestalk asymmetrically elongate conical; abdomen with scattered spines ventrally between the ovisac band and the anterior and posterior transverse bands of spines ..... *longipes* Hempel.

pp. Eyestalk rounded conical; abdomen with only the normal 4 narrow transverse bands of spines within the ovisac band ..... *praelonga* Douglas.

kk. Ovisac band inclosing 5 definite transverse spine bands, these all comparatively wide; chitinized thickening on head elongate, nearly linear ..... *artemisiae* Cockerell.

jj. Without dermal chitinization; ovisac band inclosing at least 5 more or less distinctly developed transverse spine bands; spiracular characters varying.

q. Apparently with only 4 pairs of abdominal spiracles; size small, length as mounted about 1.25 mm.; dorsal spine bands complete; ventral abdominal bands narrow; antennae 8-segmented .. *minor*, new species.

qq. With 7 or 8 pairs of abdominal spiracles; size larger, 2 mm. or more; other characters varying.

r. With 7 pairs of abdominal spiracles; eyestalk flattened, asymmetrically conical; inner margins of pore bands of anal ring not angulate posteriorly and only indistinctly so anteriorly; antennae normally 8-segmented; with a more or less distinct bare dermal area between dorsal and marginal spine clusters and secretion tufts, at least on the thorax.

s. Exposed area of derm between marginal and dorsal spine clusters much broader than the transverse width of the corresponding dorsal spine clusters; secretion corresponding, leaving wide exposed areas between dorsal and marginal tufts and between dorsal tufts; superficial appearance similar to that of *insignis* ..... *monticola* Cockerell.

ss. Exposed area of derm between marginal and dorsal spine clusters much narrower, at most not so wide as width of corresponding dorsal spine bands; dorsal secretion at most leaving only a narrow exposed band between it and marginal secretion.

t. Exposed longitudinal bands evident on anterior portion of body only, the spine bands on most of the abdominal segments complete and continuous to the corresponding marginal spine clusters; dorsal secretion more or less obviously agreeing with spine arrangement ..... *pseudograminis*, new species.

tt. Exposed longitudinal bands practically complete, except at the posterior apex of the body, most of the abdominal spine bands terminating far from the corresponding marginal spine clusters; dorsal secretion more or less obviously agreeing with spine arrangement ..... *graminis* Tinsley.

rr. With 8 pairs of abdominal spiracles; eyestalk usually distinctly conical rounded, neither flattened nor strongly asymmetrical; dorsal spine bands complete, broad at ends and only narrowly separated from corresponding marginal spine clusters; inner margins of pore bands of anal ring distinctly and sharply angulate anteriorly and posteriorly, or, if not so, antennae normally 7-segmented; dorsal secretion completely covering body in heavy tufts in perfect specimens.

u. Spines of the clusters, particularly dorsally, elongate, slender, slightly, but fairly distinctly, swollen at apices; dorsal and ventral abdominal spine bands very broad, the latter 6 in number; ovisac band with only a single row of scattered disk pores along inner margin ..... *cheilanthi* Tinsley.

uu. Spines mostly stouter, tapering uniformly from bases to rounded apices, not clavate; dorsal and ventral spine bands narrower, or at least constricted more or less distinctly at some place in their length; usually with 5 transverse ventral abdominal spine bands; ovisac band varying but not usually as described above.

v. Antennae normally 7-segmented; size smaller, 2–2.25 mm.

w. Anal ring with chitinized lateral wings, and, beyond these, a few short conical spines on each side; anterior section of ovisac band with a row of pores 2 to 3 deep along and within its outer (anterior) margin, and with a single row of pores of the same diameter along the inner (posterior) margin; eyestalk conical-rounded ..... *lasiorum* Cockerell.

ww. Anal ring without wings, conical spines on each side wanting; anterior section of ovisac band with a single row of pores along the outer (anterior) margin, and another single row, with each pore about half as large as the adjacent pores, along the inner (posterior) margin; eyestalk tuberculate, with flattened base ..... *olivacea* Cockerell.

vv. Antennae normally 8-segmented; size larger, about 3 mm. or more.

x. Ovisac band with only a single row of pores, not very distinct in one species, along the inner (posterior) margin of anterior median section; spine collars around openings of thoracic spiracles evident, but not prominently developed.

y. First antennal segment at least one-fifth wider than long, the inner margin, at most, slightly curved; row of pores along inner margin of ovisac band conspicuous ..... *boliviana*, new species.

yy. First antennal segment at least one-fifth longer than wide, the inner margin rather distinctly gibbous; row of pores along inner margin of ovisac band inconspicuous and in some sections apparently wanting ..... *grandis* Hempel.

xx. Ovisac band with a row of pores 3 or more wide along the posterior margin or through the posterior third of the anterior section; spine collars around the thoracic spiracular openings conspicuously developed.

z. Anterior median section of ovisac band with numerous pores through its posterior half, as well as a single row along the anterior margin; eyestalk usually without traces of a lateral tubercle; fourth antennal segment usually a little shorter than the fifth ..... *solidaginis* Sanders.

zz. Anterior section of ovisac band with a pore band about 3 deep only, and running along the posterior margin only, not penetrating the spine band to any marked extent; eyestalk usually with a more or less fully developed and often conspicuous lateral tubercle; fourth antennal segment usually but not always distinctly longer than the fifth ..... *urticae* (Linnaeus)

ORTHEZIA AMERICANA (WALKER)

REFERENCE.—Walker, 1852, Cat. Brit. Mus. Homoptera 4: 1091.

The identity of this species is at present unknown, and from Walker's extremely brief description it can not be recognized as any one of the North American species discussed in this paper. It is highly probable that all of the older American distribution records for this species, possibly excepting the original one, refer to what we now know as *Orthezia solidaginis* Sanders. These records include Lower Canada, New York, Ohio, and Iowa. The species was originally described from "North America."

In recent correspondence Mr. Frederick Laing of the Natural History Division of the British Museum has supplied information to the effect that the type of *Orthezia americana* is neither present in the British Museum, nor, so far as is known, in any other museum in Great Britain. Since this type apparently no longer exists, and since the species can not be placed from the original description, it seems best to set it aside as a wholly unrecognizable species and to eliminate it from further consideration in connection with the genus *Orthezia*.

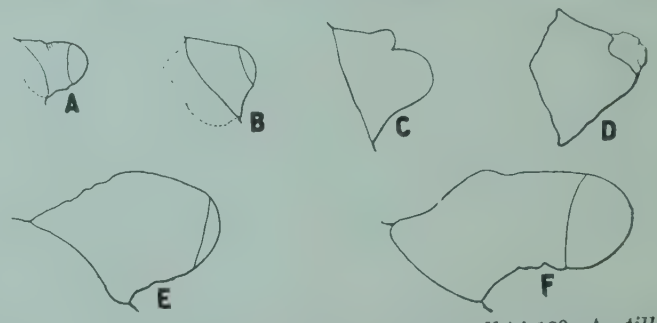


FIG. 4.—*Orthezia* spp., profiles of eyestalks, all × 120: A, *tillandsiae;* B, *ultima;* C, *urticae;* D, *varipes;* E, *cataphracta;* F, *occidentalis*

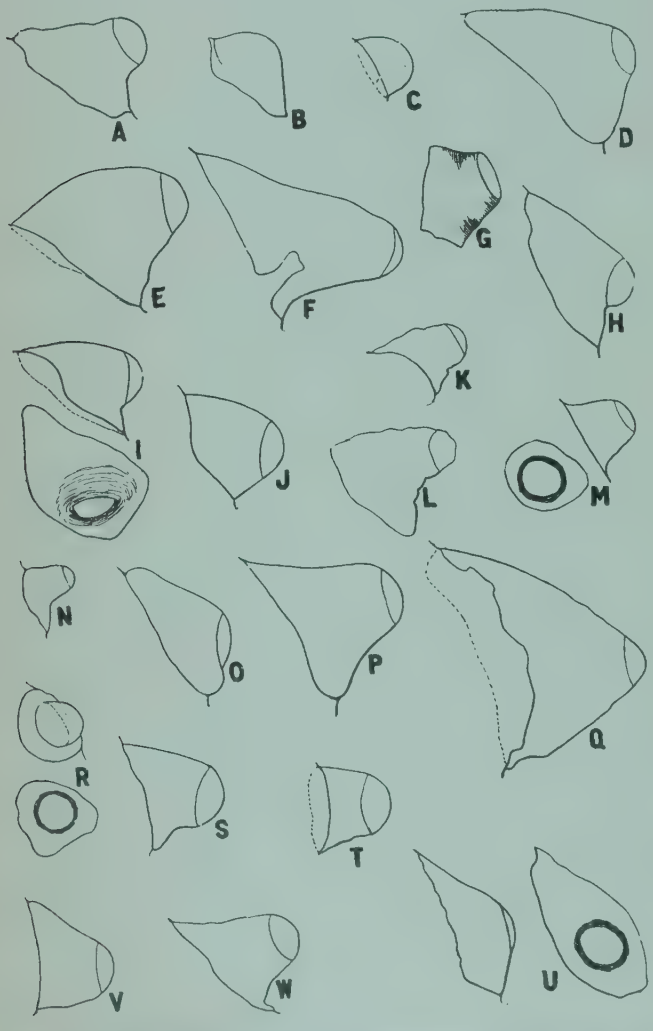


FIG. 3.—*Orthezia* spp., profiles of eyestalks, all × 120: A, *annae;* B, *artemisiae;* C, *balloui;* D, *boliviana;* E, *cheilanthi;* F, *caudata;* G, *galapagoensis;* H, *garryae;* I, *graminis;* J, *insignis;* K, *lasiorum;* L, *longipes;* M, *mexicana;* N, *minor;* O, *monticola;* P, *nigrocincta;* Q, *nuda;* R, *olivacea;* S, *praelonga;* T, *praelonga;* U, *pseudograminis;* V, *solidaginis;* W, *sonorensis*

ORTHEZIA ANNAE COCKERELL

Figs. 3, A; 5, A; 7, A; and 8; Pl. 1, A

REFERENCE.—Cockerell, 1893, Ann. and Mag. Nat. Hist. (6) 12: 403–404.

ADULT FEMALE.—Appearing approximately circular, covered dorsally with white secretion, this in a longitudinal row of fairly high tufts occupying the median third of the body, in short slightly curved tufts along the anterior and lateral margins, in considerably longer, fingerlike tufts around the posterior margin, and in low transverse ridges between the median and marginal tufts; length of ovisac variable according to development, but sometimes more than twice the length of the body, the upper surface divided into broad longitudinal plates; the body ventrally completely covered with secretion except around the insertions of the appendages; all body secretion rather fragile and easily mutilated; length as mounted on slide about

2.5 millimeters; width about 2.25 millimeters; body broad, tapering more or less distinctly anteriorly, but with the head broadly rounded; derm membranous except for a small, but fairly distinct, chitinized thickening along the outer margin of each half of the anal ring; antennae normally 8-segmented, average lengths of the segments of one in microns as follows: I, 143; II, 129; III, 161; IV, 111; V, 136; VI, 96; VII, 89; VIII, 171; eyestalk broad at base, tapering strongly to beyond middle, with apex short, cylindrical, rounded at tip; legs characteristic for the genus, tarsal claw with 2 indistinct denticles; beak about 268 microns long and 239 microns wide, 1-segmented but with a faint suggestion of a joint about one-third of the length from the base; each of the thoracic spiracles with a loose double band of very short, bluntly triangular spines around its opening, these spines differing conspicuously from the normal body type; with 8 pairs of much smaller, simple, tubular, abdominal spiracles; derm pores of the quadrilocular disk type only, varying slightly in size and appearance and occurring chiefly in the dorsal and ventral abdominal regions, most abundant along and within the inner margin of the ventral ovisac band, penetrating this in some places as much as one-third of its total width and forming a band 3 or 4 deep within the ovisac band; body spines arranged in bands and clusters approximately as shown in the figure, the median dorsal abdominal bands strongly attenuated laterally, the inner 15 to 20 spines of each of the 2 clusters immediately anterior to the anal ring conspicuously shorter than the remaining spines in these and the other bands; dorsal spines and ventral thoracic spines approximately the same in size and shape, more or less distinctly curved, tapering from base to apex; spines in the broad ovisac band and the 5 ventral rows inclosed by this band plainly, but not conspicuously, smaller and more slender than the dorsal spines, lateral and posterior portions of ovisac band more or less distinctly divided into several sections by transverse clear spaces each bearing an irregular row of disk pores; anal ring only fairly well developed as compared with some other members of the genus, with a lateral chitinized wing on each side, the chitinization anteriorly and posteriorly indistinct or wanting and the pore bands on each terminating well short of the median longitudinal line, particularly posteriorly, and not angulate within; with the usual 6 anal ring setae.

LARVA.—Stout oval, tapering slightly at each end, length when mounted 410 microns; width 311 microns; antennae 6-segmented, apical much the longest; beak relatively large, stout conical; legs not unusual, tarsal claw with 2 fairly distinct denticles; body spines both dorsally and ventrally rather abundant, arranged in transverse rows, those on the dorsal surface interrupted medially and again about half-way between the middle line and margin, those of venter apparently continuous, the inner 2 to 4 spines of each of the bands just anterior to the anal ring short triangular, distinctly differing in size and shape from the remainder of the body spines; anal ring much as in adult, except for reduction

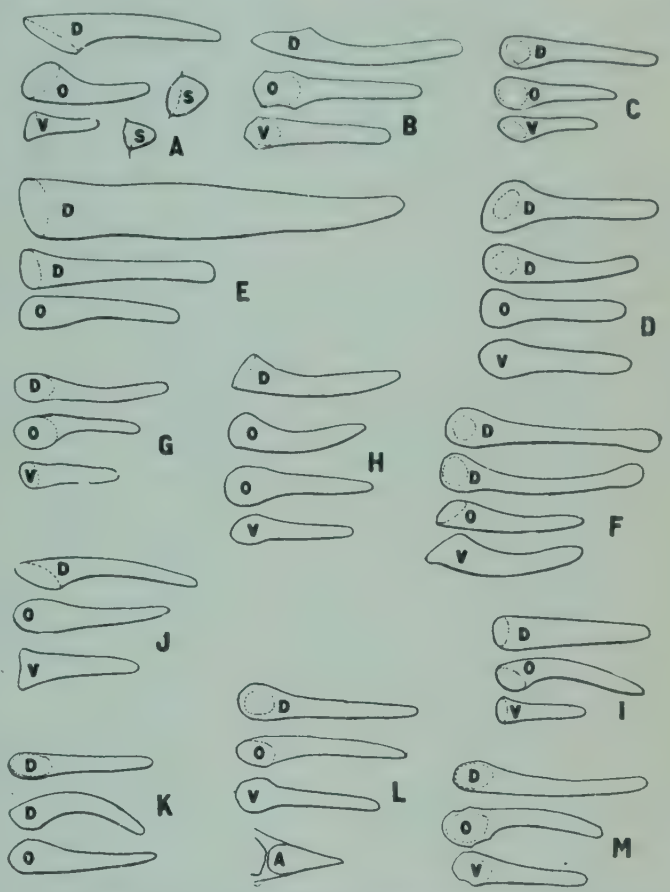


FIG. 5.—*Orthezia* spp., outlines of average body spines (d = dorsal, o = ovisac band, v = ventral abdominal, s = spiracular collar, a = near anal ring), all × 720: A, *annae;* B, *artemisiae;* C, *balloui;* D, *boliviana;* E, *caudata;* F, *cheilanthi;* G, *galapagoensis;* H, *garryae;* I, *graminis;* J, *grandis;* K, *insignis;* L, *lasiorum;* M, *longipes*

and absence of the lateral chitinized wings.

This species has been redescribed from the following material: Las Cruces, N. Mex., on *Atriplex canescens* (Chenopodiaceae), coll. T. D. A. Cockerell (type); and Arizona on Chenopodium (Chenopodiaceae), coll. C. H. T. Townsend.

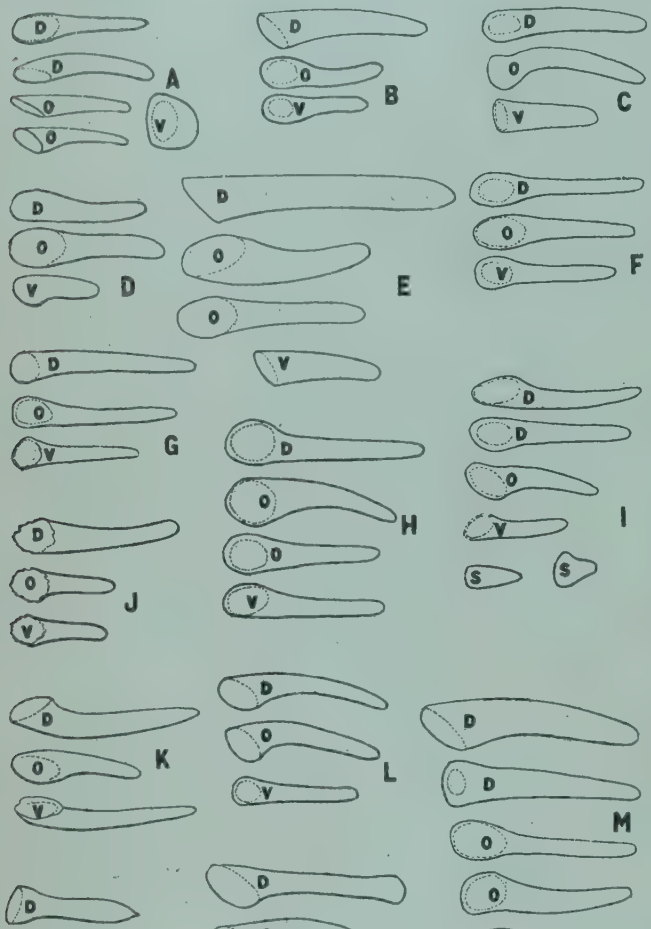


FIG. 6.—*Orthezia* spp., outlines of average body spines (letter designations as in fig. 5), all × 720: A, *mexicana;* B, *minor;* C, *monticola;* D, *nigrocincta;* E, *nuda;* F, *olivacea;* G, *praelonga;* H, *solidaginis;* I, *sonorensis;* J, *tillandsiae;* K, *varipes;* L, *ultima;* M, *urticae;* N, *cataphracta;* O, *occidentalis*

ORTHEZIA ARENARIAE VAYSSIÈRE

REFERENCE.—Vayssière, 1924, Bul. Soc. Ent. France No. 2: 28–29, fig. 1.

This species, described since the completion of the present paper, as characterized by the author, is evidently very closely related to *O. urticae* (L.). It was collected on the Djebel Tachdirt, Morocco, on *Arenaria pungens* (Caryophyllaceae). No specimens have been available for comparative study, and it has consequently not been possible to place the species precisely among those included in the key.

ORTHEZIA ARTEMISIAE COCKERELL

Figs. 3, B; 5, B; and 9; Pl. 1, B

REFERENCE.—Cockerell, 1898, Canad. Ent. 30: 19–20.

Only a single badly mutilated adult female and a few immature specimens of this species have been available for examination from the type material, and from these it has not been possible to place it with certainty. Professor Essig has very kindly supplied specimens which he has collected at Santa Paula, Calif., on *Artemisia californica* (Compositae) which appear, so far as comparison is possible, to be identical with the type, and the species has been included in the key and figured mostly on the basis of a study of Professor Essig's specimens. An extended redescription as well as the precise placing of the species within the genus must be deferred until satisfactory topotype material can be obtained. The species was originally described from Embudo, N. Mex., on Artemisia (Compositae), collected by T. D. A. Cockerell, October, 1897.

ORTHEZIA BALLOUI, NEW SPECIES

Figs. 3, C; 5, C; 7, B; and 10; Pl. 1, C

ADULT FEMALE.—Approximate length of body with all secretion, including posterior marginal tufts, 1.6 millimeters; width 1 millimeter; total length of well-developed female with ovisac 4.4 millimeters; body, except for a tiny quadrate bare spot, completely covered dorsally and ventrally with tufts of secretion, these more or less distinctly arranged in the usual marginal and lateral tufts; posterior marginal tufts of secretion very long, sometimes nearly as long as the body; average length of body as mounted 1.2 millimeters, width 1 millimeter, very broad oval, posterior apex somewhat flattened, anterior very slightly narrowed; derm membranous, except for 4 pairs of prominent, rectangular,

chitinized plates, these replacing the outer halves of the dorsal spine bands on 4 of the anterior abdominal segments, differing, in the possession of these chitinous plates, from all the previously described species of Orthezia and resembling in this respect only the species *tillandsiae*, but differing lengths of the segments of one in microns as follows: I, 82; II, 75; III, 107; IV, 75; V, 82; VI, 79; VII, 68; VIII, 154; eyestalk small, tuberculate rounded; beak conical, 1-segmented; legs characteristic for the genus, rather slender, tarsal claw with 2 comparatively prominent denticles on the inner

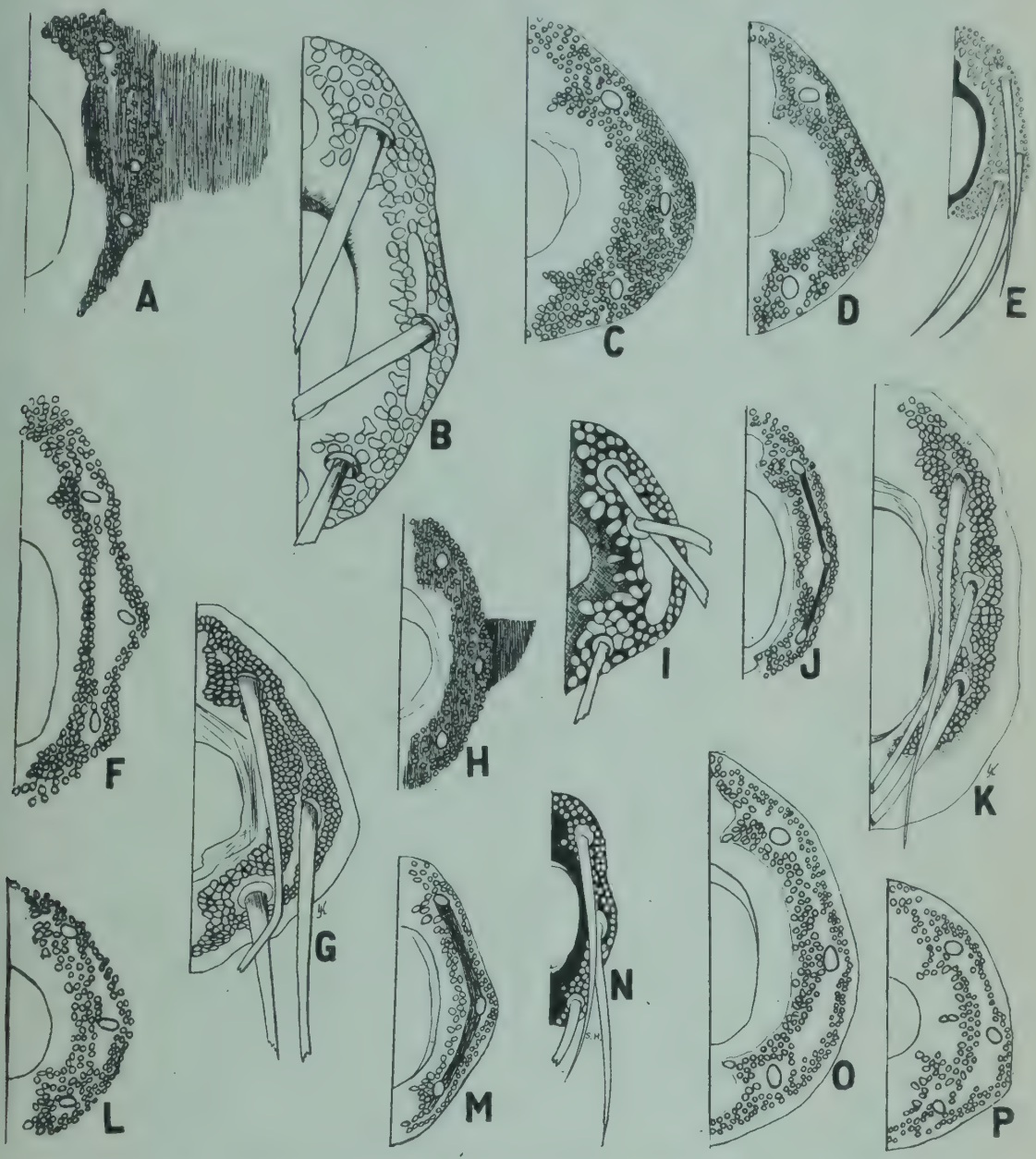
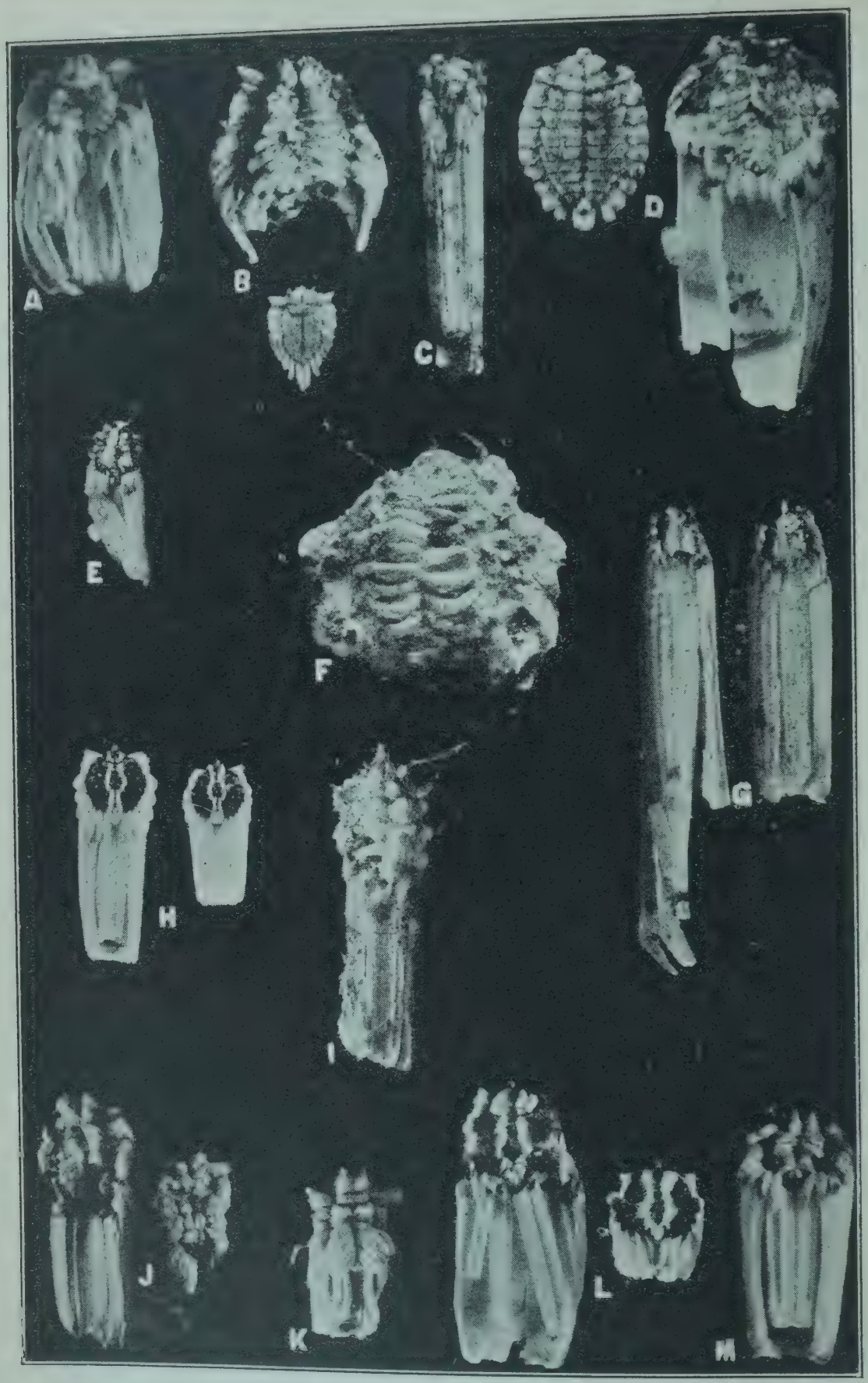


FIG. 7.—*Orthezia* spp., right half of anal ring of certain species, all × 217, except B and I, which are × 510: A, *annae;* B, *balloui;* C, *boliviana;* D, *cheilanthi;* E, *galapagoensis;* F, *garryae;* G, *grandis;* H, *lasiorum;* I, *minor;* J, *nigrocincta;* K, *nuda;* L, *olivacea;* M, *praelonga;* N, *tillandsiae;* O, *urticae;* P, *cataphracta*

obviously from the latter in that the plates are much more conspicuous, much better developed, and interrupt the dorsal spine bands instead of merely occupying a position between the outer sections of such bands; antennae normally 8-segmented, the face; thoracic spiracles characteristic for the genus, each opening in a spine cluster but without definite spine collar around the opening; with, so far as can be determined, 4 pairs of long tubular abdominal spiracles; derm pores of the usual quadrilocular disk



Scale Insects of the Subfamily Ortheziinae Plate I

A, *Orthezia annae*, adult female; B, *O. artemisiae*, mutilated adult and immature females; C, *O. balloui*, adult female, secretion mutilated; D, *O. cheilanthi*, mutilated adult and preadult females; E, *O. galapagoensis*, adult female, secretion mutilated; F, *O. grandis*, adult female, ovisac wanting; G, *O. graminis*, adult females; H, *O. insignis*, adult females (photo by J. G. Sanders); I, *O. longipes*, adult female, attacked by fungus; J, *O. mexicana*, adult females, secretion badly mutilated; K. *O. minor*, adult female; L, *O. monticola*, adult females, secretion slightly mutilated; M, *O. nigrocincta*, adult female

ype, occurring both dorsally and ventrally, most abundant ventrally in the posterior abdominal region; derm with occasional setae both dorsally and ventrally, these most abundant ventrally in a cluster just anterior to the genital opening; derm with the usual 11 marginal and 10 dorsal clusters of spines, the dorsal spine bands wide, continuous from the median line to the marginal clusters, except for the interruptions formed by the chitinized plates on the abdomen, as already described; ventral ovisac band wide, made up of closely crowded spines with a few disk pores along the anterior margin, the band inclosing 3 loose transverse rows of spines, the anterior several spines wide, the 2 posterior little more than irregular single rows, all continuous and reaching nearly to the ovisac band at each end; anal ring elongate oval, each half with the usual inner and outer pore bands with the inner margin distinctly angulate anteriorly and posteriorly, and the bands of each half joined anteriorly but more or less distinctly separated behind; with the usual 6 anal ring setae.

FIG. 9.—*Orthezia artemisiae:* Adult female, body, dorsal and ventral; × about 31.

This species has been described from five mounted and two or three unmounted adult females recently forwarded for determination by C. H. Ballou, Officina de Sanidad Vegetal, Havana, Cuba. The specimens were collected at Pico Turquino, Oriente Province, Cuba, on *Palicourea crocea* (Rubiaceae), July 22, 1922, by C. H. Ballou and S. C. Bruner under No. 364.

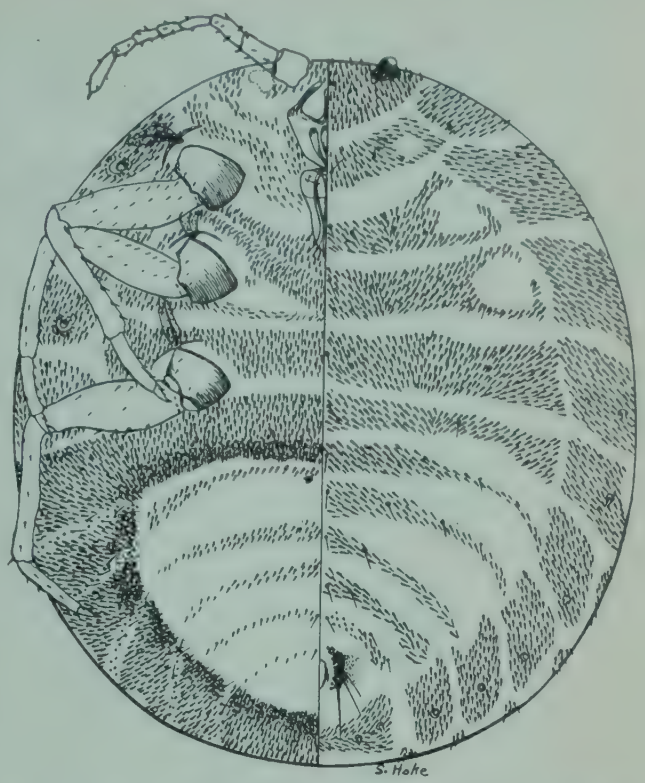


FIG. 8.—*Orthezia annae:* Adult female, body, dorsal and ventral; × about 31.

The types are in the U. S. National Collection of Coccidae.

ORTHEZIA BOLIVIANA, NEW SPECIES

FIGS. 3, D; 5, D; 7, C; and 11

ADULT FEMALE.—Stout oval, length with secretion little more than 3 millimeters, width about 2.5 millimeters, very slightly narrowed anteriorly; ovisac short, average length about 2.5 millimeters, presumably completely covered both dorsally and ventrally with white secretionary plates, and these, in a portion of the dorsal area at least, forming overlapping scalelike plates similar to those of *cheilanthi*, but this secretion much too incomplete for description in the specimens available for examination; length as mounted about 3 millimeters, width 2.5 millimeters, broad oval, very slightly narrowed anteriorly; derm membran-

ous; antennae normally 8-segmented, rather short and stout, lengths of the segments of one in microns as follows: I, 107; II, 100; III, 85; IV, 61; V, 61; VI, 57; VII, 60; VIII, 168; spine, 25; eyestalk heavily chitinized, asymmetrically flat conical, apex rounded; legs characteristic for the genus, inner face of tarsal claw usually with 2 tiny denticles; beak rather long triangular, 1-segmented, with a faint suggestion of a joint near the base; thoracic spiracles characteristic for the genus, each opening into a pore cluster, each opening surrounded by a fairly distinct collar of spines; with 8 pairs of tubular abdominal spiracles; derm pores of the usual quadrilocular disk type, occurring both dorsally and ventrally, those of the dorsum usually more heavily chitinized than the ventral pores; with

FIG. 10.—*Orthezia balloui:* Adult female, body, dorsal and ventral; × about 31

occasional setae both dorsally and ventrally, these most abundant in a transverse cluster anterior to the genital opening ventrally; derm spines arranged in the usual 11 marginal and 10 dorsal clusters, the dorsal bands transverse, broad, extending close to the corresponding marginal clusters; ventral ovisac band wide, made up of numerous spines, these more crowded toward the inner margin, and along the latter a single, irregular row, sometines more or less interrupted, of disk pores; band inclosing 5 transverse rows of spines, these terminating laterally before attaining the ovisac band, and with the anterior several spines wide; anal ring short oval, with the pore bands on each half very broad and the inner and outer halves completely joined to form a single band, except, at most, for a very short distance posterior to the insertion of the middle seta, each band joined to the other at the ends and angulate within anteriorly and posteriorly as in *urticae*.

LARVA.—Not displaying any obvious peculiarities; antennæ 6-segmented; anal ring much as in adult except for reduction; spines arranged in transverse segmental rows; dorsal spines crowded into marginal and submedian clusters on each half of the body.

This species has been described from five mounted and a few unmounted adult females and three mounted larvae, all collected at Guaqui, Bolivia, among roots of "Vareta," by W. R. Allen, March 7, 1919. The specimens were received for study from Prof. J. G. Needham of Cornell University, Ithaca, N. Y.

The types are in the U. S. National Collection of Coccidae.

ORTHEZIA CAUDATA FERRIS

Figs. 3, F; 5, E; and 12

REFERENCE.—Ferris, 1921, Stanf. Univ. Pubs., Biol. Sci. 1: 71, fig. 1.

ADULT FEMALE.—No specimens available for description of external apperance (see reference cited for this); length, as mounted, 3 millimeters, width about 2.75 millimeters, broad oval, tapering somewhat anteriorly; derm membranous except for appendages and faint linear longitudinal median stripe running back from the anterior margin on the dorsal surface for a little more than the width of the anterior marginal spine cluster; antennae normally 8-segmented, lengths in microns for one as follows: I, 178; II, 143; III, 186; IV, 146; V, 161; VI, 161; VII, 143; VIII, 232; inner face of first segment strongly bulging; eyestalk elongate conical, heavily chitinized; legs characteristic for the genus, elongate, rather slender; tarsal claw with 3 to 4 slight denticles on its inner face; beak elongate conical, 1-segmented, with a suggestion of a joint about one-third of its length from base; thoracic spiracles not unusual, external opening surrounded by a very loose circle of quadrilocular disk pores but without a distinct collar of spines; with 8 pairs of long tubular abdominal spiracles; derm pores of the usual quadrilocular disk type only, these fairly well chitinized, apparently vary-

ing only slightly in size and quite abundant as compared with many other species of the genus, occurring both dorsally and ventrally, particularly in the posterior portion of the area enclosed by the ovisac band, occurring here in crowded transverse bands of less heavily chitinized, somewhat smaller pores; normal body spines arranged in groups about as shown in figure, these present in 11 marginal clusters including the anterior and 10 dorsal transverse bands on each half of the body; conspicuously stouter and heavier body spines interspersed through these clusters, about as shown in figure, but the arrangement apparently more or less variable; ovisac band fairly broad, made up of numerous spines, with a considerable number of disk pores scattered through the whole breadth of the band, but more numerous on the anterior and posterior margins, indistinctly interrupted laterally and posteriorly by narrow clear strips; ovisac band inclosing, besides the numerous pores already mentioned, apparently 4 fairly distinct transverse bands of rather elongate, slender spines; anal ring well developed, characteristic for the genus, with the inner margin of the band of numerous pores on each half distinctly but not conspicuously angulate; ring normally bearing 6 fairly long setae.

This species has been redescribed from a single adult female from Todos Santos Lower California, on *Encelia palmeri* (Compositae) collected by G. F. Ferris, August, 1917 (paratype). There are no additional published records of its occurrence.

FIG. 11.—*Orthezia boliviana*: Adult female, body, dorsal and ventral; × about 31

ORTHEZIA CHEILANTHI TINSLEY

Figs. 3, E; 5, F; 7, D; and 13; Pl. 1, D

REFERENCE.—Tinsley, 1898, Canad. Ent. 30: 12–13, fig. 1.

ADULT FEMALE.—Length of dried specimens with secretion about 3.5 millimeters, width approximately the

same; ovisac about 4 to 4.5 millimeters long, nearly as wide as the body, narrowed near the posterior apex, composed of a solid dorsal plate with lateral keels and longitudinal striations, a broad, curved, transversely strongly rounded, nearly smooth ventral plate and, between these two, 3 more or less distinct elongate ribs; body secretion dorsally arranged in the usual marginal plates, these rather short anteriorly but becoming longer, more strongly curved, flattened and somewhat pointed toward the posterior end of the body, the penultimate pair from one and a half to two times the length of any of the others; secretion covering the disk of the dorsum, instead of being arranged in the usual 10 pairs of definite and distinct plates, with only the inner portion of each of these plates definitely formed, the remainder of the secretion made up of a number of overlapping scalelike plates, producing an appearance rather different from that usually found in the genus; length of body as mounted normally about 3.5 millimeters, width about 2.5 to 3 millimeters, nearly oval, somewhat pointed anteriorly; derm membranous; antennæ normally 8-segmented, lengths of segments of one in microns as follows: I, 161; II, 100; III, 150; IV, 93; V, 89; VI, 85; VII, 89; VIII, 186; eyestalk stout, rounded conical, somewhat curved; legs apparently not unusual for the genus, although no complete examples available for examination; beak stout conical, 1-segmented, without indication of a further division; thoracic spiracles normal, the external opening of each surrounded by a collar of spines differing distinctly but not conspicuously in shape and size from the remaining body spines; with 8 pairs of long tubular, submarginal abdominal spiracles; derm pores of the usual quadrilocular

FIG. 12.—*Orthezia caudata:* Adult female, body, dorsal and ventral; × about 31

disk type only, occurring both dorsally and ventrally, most abundant ventrally in the posterior portion of the area inclosed by the ovisac band, only rarely heavily chitinized and nearly always with a distinctly wider marginal area around the 4 loculi than is usual for the genus; body spines relatively

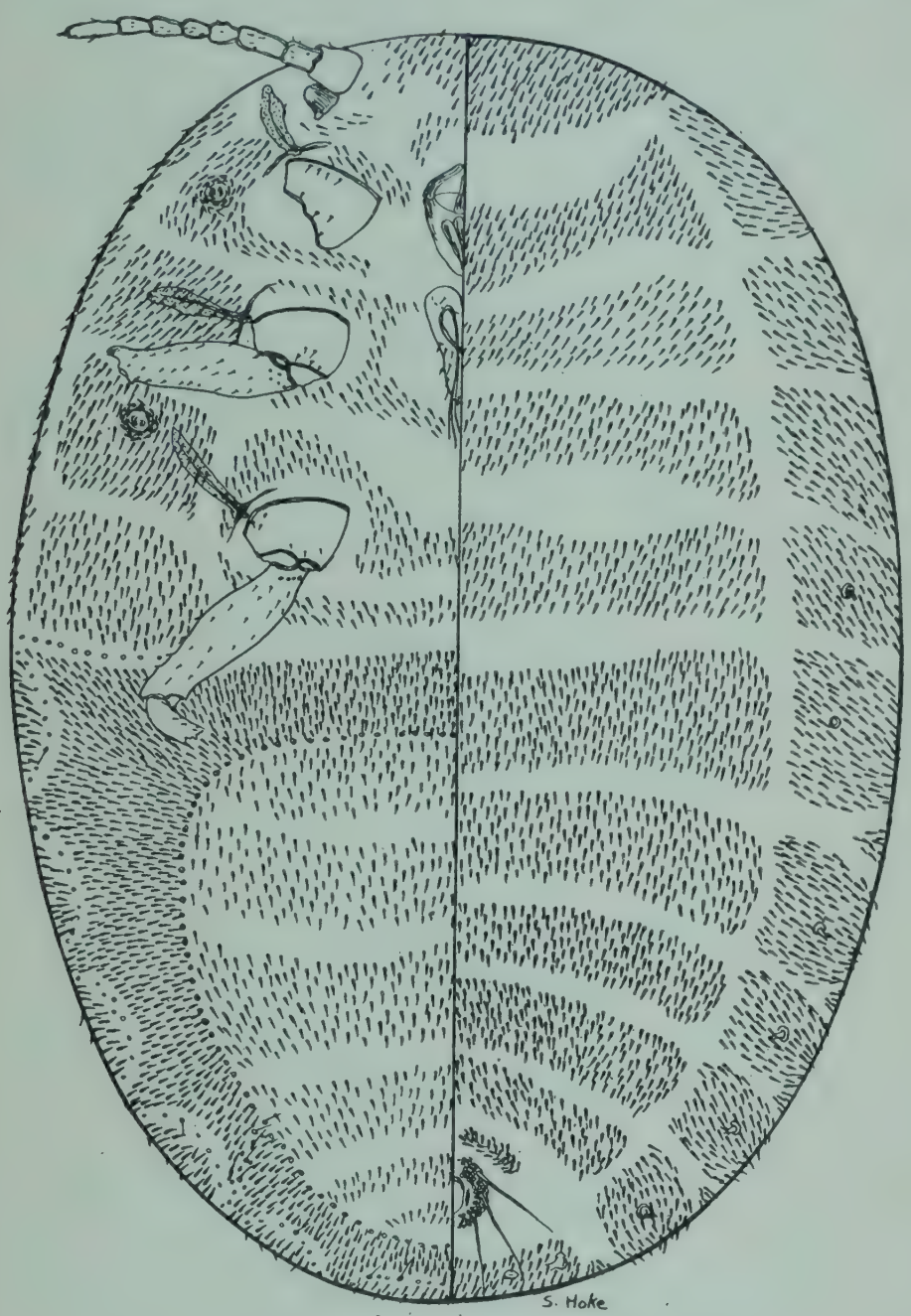


FIG. 13.—*Orthezia cheilanthi:* Adult female, body, dorsal and ventral; × about 31

elongate, slender, normally only slightly tapering and with apices slightly but distinctly swollen in contrast to the usual rather strongly tapering, apically rounded or pointed spines occurring in most species in the genus; spines arranged in the usual 11 marginal and 10 submarginal clusters on each half dorsally but, so far as can be determined, not broken up into small groups or clusters to correspond precisely with the scalelike plates of secretion; ovisac band rather broad, interrupted only once in each half, composed only of spines, with a single row of disk pores along its inner margin, inclosing, so far as can be determined, 6 broad transverse bands of rather loosely grouped spines, these, in combination, almost completely filling the space surrounded by the ovisac band; anal ring apparently not unusual for the genus, with the usual 6 rather long tapering setæ, the inner margin of the pore band on each half sharply angulate anteriorly and posteriorly, the pore bands sometimes united or nearly so at their apices.

This species has been redescribed from the following material: Organ Mts., N. Mex., on *Cheilanthes fendleri* (Polypodiaceae, Order Filicales), collected by J. D. Tinsley, August, 1897 (type). There appears to be no subsequent record of its continued existence or wider distribution.

ORTHEZIA EDWARDSII ASHMEAD

REFERENCE.—Ashmead, 1888, Canad. Ent. 20: 203–204.

Only the male of this species has been described and an examination of the description shows clearly that the species can not be assigned to the genus Orthezia, but, as was suggested by Professor Cockerell, it is probably the male of a pseudococcine form, and very likely the male of a species of Puto or Ceroputo. The species was described from California.

ORTHEZIA GALAPAGOENSIS KUWANA

Figs. 3, G; 5, G; 7, E; and 14; Pl. 1, E

REFERENCE.—Kuwana, 1902, Jour. N. Y. Ent. Soc. 10: 28–29.

ADULT FEMALE.—External appearance most nearly resembling that of *graminis* and *nigrocincta*, in that there is a distinct exposed longitudinal derm band on the dorsum on each side between the median and marginal tufts; body of female, as mounted on slide, about 1.5 millimeters long by 1 millimeter wide; derm membranous throughout except for median longitudinal anterior chitinized stripe extending posteriorly from the front margin of the head and varying somewhat in extent and conspicuousness; antennae normally 8-segmented, measurements of segments of one in microns as follows: I, 70; II, 79; III, 100; IV, 107; V, 89; VI, 93; VII, 82; VIII, 186; spine, 25; eyestalk stout conical, apex more or less distinctly flattened; legs not unusual for the genus, but long in proportion to the size of the body, in this particular resembling the proportions in *insignis* and *praelonga*, tarsal claw slender, nearly straight, inner face with 2 or 3 indistinct denticles on apical half; beak stout conical, apex rounded, without traces of segmentation at any point, with the usual 2 pairs of thoracic spiracles, the opening of each of these within a cluster of spines, but surrounded neither by a distinct spine collar nor by differentiated spines of any kind; with 7 pairs of long tubular abdominal spiracles; derm pores of the quadrilocular disk type only, these scattered sparingly over the body, most abundant in the inner portion of the ovisac band, each of

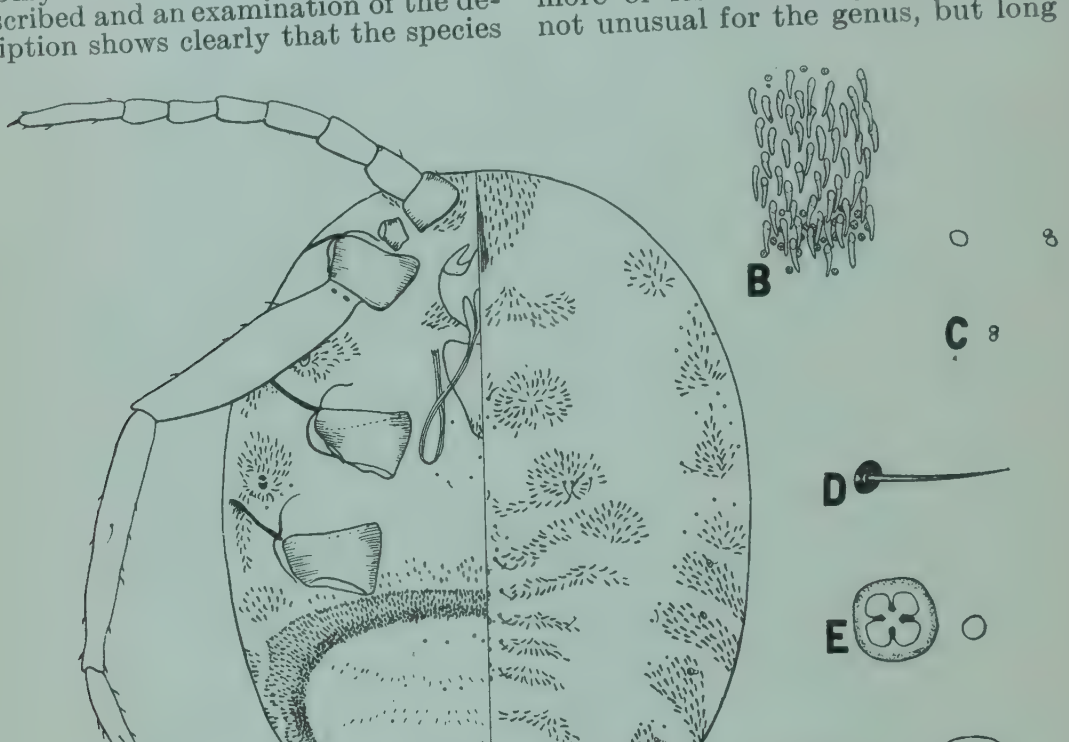


FIG. 14.—*Orthezia galapagoensis*, adult female: A, Body, dorsal and ventral, × about 55; B, section through median area of anterior portion of ovisac band, × 220; C, simple derm pores, × 509; D, body seta, × 500; E, dorsal quadrilocular pore, accompanied by simple pore, × 1,500; F, ventral quadrilocular disk pore, × 1,500; G, disk pore from ovisac band, × 1,500

hose on dorsum, at least, usually accompanied by one or two tiny clear circles; body spines of the usual sort, and arranged in the usual 10 dorsal and 11 marginal clusters, approximately as shown in figure; dorsal spines longest, straight or slightly curved, ventral abdominal spines the shortest and stoutest; body with setae occasionally both dorsally and ventrally, these longer and larger dorsally; ovisac band rather narrow, made up as usual of crowded spines, a single row of scattered disk pores about 3 to 4 deep along and within the inner margin, band inclosing 4 transverse rows of scattered spines; anal ring not unusual for the genus, the inner margin of the pore bands indistinctly angulate anteriorly but without such angulation behind, ring bearing the usual 6 setae; posterior portion of the ventral abdominal area with some small clear disks, possibly ventral cicatrices, in addition to a number of the usual disk pores.

This species has been redescribed from two specimens on "*Scalesia microcephala* 254, Tagus Cape Albemarle I. Type L. 375–S. 1 Entomological Lab. Stanford University S. I. K. 1901" remounted and very kindly loaned for study by Prof. G. F. Ferris of Stanford University. Additional specimens, recently reported on by the writer, collected by Dr. Wm. Beebe on *Heliotropinum parviflorum* (Borraginaceae) and on *Bursara graveolens* (Burseraceae) in the Galapagos Islands, have also been examined.

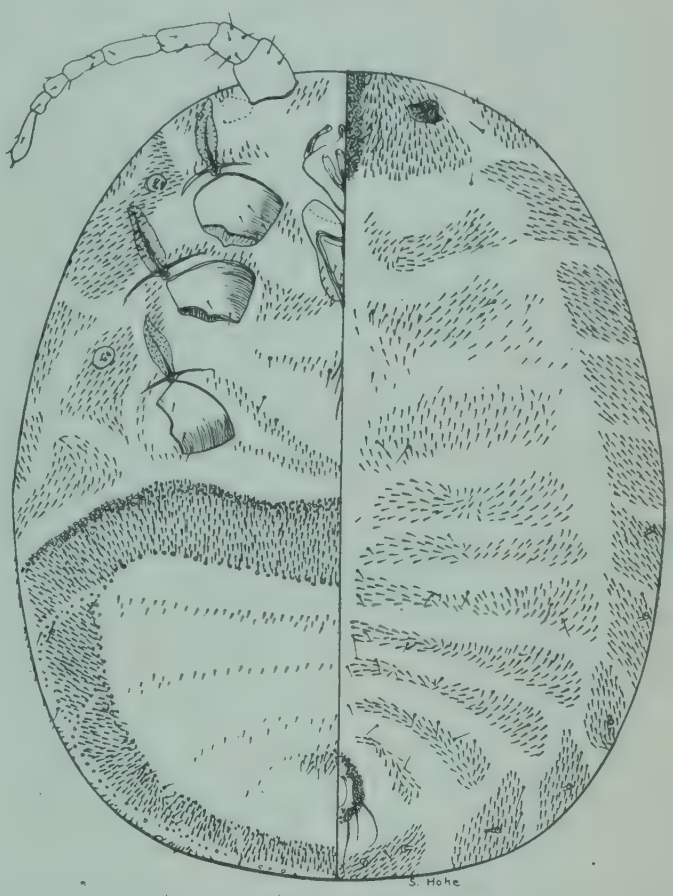


FIG. 15.—*Orthezia garryae:* Adult female, body, dorsal and ventral; × about 31

ORTHEZIA GARRYAE COCKERELL

Figs. 3, H; 5, H; 7, F; and 15

REFERENCE.—Cockerell, 1898, Ann. and Mag. Nat. Hist. (7) 2: 401–402.

ADULT FEMALE.—No specimens available for redescription of external appearance and the following quoted from the original description: "Length about 2½ mm., with ovisac about 7 mm. Body pale pea green; ovisac strongly curved upwards, composed of ribbon-like longitudinal bands, which are contiguous, but little or not coherent; lateral dorsal area only clothed with thin meal; middle of back with a double crest of long erect white lamellae; sides with long thick curling white lamellae, the two at the beginning of the ovisac on each side very long and curving downward over the side of the ovisac; caudal lamellae very short." Length of body as mounted 2.5 mm., width about 2 mm.; broadly rounded posteriorly, tapering somewhat anteriorly; derm membranous, except for a fairly distinct dorsal median, longitudinal, somewhat chitinized band extending in from the anterior margin about the width of the anterior spine cluster; antennae normally 8-segmented, lengths of segments of one in microns as follows: I, 140; II, 125; III, 186; IV, 168; V, 143; VI, 104; VII, 96; VIII, 207; spine, 25; basal portion of eyestalk flat conical, with an angular, protruding, hemispherical, apical cap; legs not unusual for the genus, the spines on all the segments relatively more elongate and slender than in most of the species of the genus, tarsal claw with 2 or 3 fairly distinct denticles on the inner face; beak stout conical, 1-segmented, without indication of any subdivisions; thoracic spiracles characteristic for the genus, each surrounded by a spine cluster, but without a distinctly developed spine collar; with 7 pairs of long tubular, abdominal spiracles; derm pores of the usual quadrilocular disk type only, these present both dorsally and ventrally, but much more

numerous over the ventral surface, most of these with rather wide outer walls surrounding the loculi, the maximum and minimum diameters varying to a marked degree, the largest nearly twice the size of the smallest; body with setae scattered sparingly over most of the surface, but with a distinct and rather prominent transverse cluster of slender setae occurring ventrally just anterior to the genital opening; spines arranged in the usual 11 marginal and 10 dorsal clusters on each half of the body about as shown in figure, the dorsal bands narrowed medially or otherwise modified; ovisac band broad anteriorly, somewhat narrowed posteriorly, made up of spines, with a band of disk pores 2 to 3 deep along its anterior and outer margins, and a single irregular row along the inner margin of the anterior section, this broadening to an irregular row 2 or 3 deep along the inner margins of the lateral and posterior sections, band interrupted laterally and posteriorly by transverse clear areas, and inclosing, besides the disk pores already mentioned, 4 narrow transverse bands of scattered spines extending almost to the ovisac band at each end; anal ring elongate oval, the outer margin sinuate opposite the insertions of the 6 ring setae, with numerous pores, the inner margin of pore row distinctly but not prominently angulate anteriorly and posteriorly.

FIG. 16.—*Orthezia graminis*: Adult female, body, dorsal and ventral; × about 31

This species has been redescribed from the following material: Dripping Springs, Organ Mountains, N. Mex., on *Garrya wrightii* (Cornaceae), coll. T. D. A. Cockerell (type). Only one additional record has been made, the species having been identified by Cockerell from material collected at Grand Canyon, Ariz., on Fendlera (Hydrangeaceae) by E. Bethel.

ORTHEZIA GRAMINIS TINSLEY

Figs. 3, I; 5, I; and 16; Pl. 1, G

REFERENCE.—Tinsley, 1898, Canad. Ent. 30: 13–14, fig. 2.

ADULT FEMALE.—Average length of body of dried specimens about 2 millimeters, width somewhat less; broad oval, tapering somewhat anteriorly, ovisac, when fully developed, much longer than the body, attaining a length of as much as 10 millimeters; dorsally with marginal and median plates or tufts of secretion, leaving an intermediate submarginal bare band running the full length of the body and interrupted by the dorsal plates only at the posterior end of the body; all secretions fragile, lateral tufts smaller, fingerlike, posterior ones flattened and curved backwards over the ovisac, middle dorsal tufts plainly paired, closely crowded and standing nearly erect and fairly high; ovisac more or less distinctly ribbed and striate dorsally; anterior pair of median plates directed forward and appearing much larger than any of the others; body, as mounted, more or less distinctly oval, usually tapering somewhat anteriorly, length averaging a little more than 2.5 millimeters, width averaging about 2 millimeters, derm membranous except for a slight chitinized thickening on each side of the genital opening; antennae normally 8-segmented, measurements of one in microns as follows: I, 118; II, 107; III, 125; IV, 107; V, 107; VI, 96; VII, 89; VIII, 143; spine, 18; eyestalk rather flat conical, with

the eye slightly protruding; legs not unusual for the genus, fairly slender, tarsal claw with 1 or 2 indistinct denticles on the inner face; beak short, stout conical, 1-segmented, without definite indication of any subdivision; thoracic spiracles entirely characteristic for the genus, each surrounded by a spine cluster and with a fairly distinct band of spines about its opening; with 7 pairs of long tubular abdominal spiracles; derm pores of the quadrilocular disk type only, these very distinctly present in two sizes, the smaller hardly more than half the size of the larger; with a few slender setae scattered over the body surface, these more abundant in the ventral area enclosed by the ovisac band; derm spines occurring in the usual 11 marginal and 10 dorsal clusters, with a distinct and broad clear area in the derm widely separating corresponding marginal and dorsal bands; ovisac band broad and heavy, made up of numerous spines and with a considerable number of pores scattered through the inner one-half to two-thirds of the band; band inclosing 5 transverse bands of scattered spines, each band of moderate width and extending well towards but not reaching the inner margin of the ovisac band; anal ring rather elongate oval, the pore bands on each half relatively narrow, not very heavily chitinized, usually united or nearly so anteriorly, but more widely separated posteriorly, the inner margins not sharply angulate anteriorly and posteriorly as in many species of the genus, with the usual 6 tapering setae.

This species has been redescribed from the following material: Mesilla Park, N. Mex., on grass (Gramineae), coll. J. D. Tinsley, September 26–7, 1897 (type); Dona Ana, N. Mex., on grass, coll. C. H. T. Townsend, September 26, 1897. Published records for the species include Marshall Co., Kans., on *Solidago* sp. (Compositae), and Los Angeles Co., Calif., on a perennial grass. A number of specimens previously determined as *graminis* are included under *pseudograminis* subsequently described as new.

ORTHIZIA GRANDIS HEMPEL

Figs. 5, J, and 7, G; Pl. 1,F

REFERENCE.—Hempel, 1920 (received 1921), Rev. Mus. Paulista 12: 342–343, 365–367.

Through the courtesy of the describer of this species, and of the Museo Paulista, the present writer has been permitted to examine specimens from the type material, and it has been included in the key on the basis of this examination. The mount obtained from the single adult female received was unfortunately not sufficiently satisfactory to permit the preparation of a figure showing the spine groups or to permit an adequate redescription comparable to those given for other species discussed in this paper.

This species was collected near Sao Paulo, Brazil, on *Guadua distorta* Rupr. ? (Gramineae).

ORTHEZIA INSIGNIS DOUGLAS

Figs. 3, J; 5, K; 17, and 18; Pl. 1, H

REFERENCE.—Douglas, 1887, Ent. Mo. Mag. 24:95–101, 165–171, illus.

SYNONYM.—*Orthezia nacra* Buckton (see Fernald, 1903, Cat. Cocc. World, p. 34).

FIG. 17.—*Orthezia insignis:* Adult female, body, dorsal and ventral; × about 31

ADULT FEMALE.—Body almost uniformly oval; secretion very much reduced, occurring laterally in the usual fashion, except that the anterior plates are much reduced in size and the posterior marginal plates rather elongate and slender, but present dorsally only as 2 median longitudinal rows of small tufts, closely approximated anteriorly and posteriorly, but somewhat separated about the middle of the body, leaving a broad, dull-green bare area running the full length of the body on each side between the marginal and median rows, and a much smaller, oval, similarly bare area medially; apices of anterior dorsal plates also separated, leaving another median bare area at the apex of the head; ovisac variable in length, somewhat narrowed posteriorly, more or less distinctly ribbed dorsally and sometimes nearly twice the

length of the body; body of female, as mounted, somewhat variable in size, but in well developed specimens averaging about 1.5 millimeter in length by 1 to 1.25 millimeter in width; derm membranous; antennae normally 8-segmented, measurements of the segments of one in microns as follows: I, 100; II, 68; III, 82; IV, 79; V, 79; VI, 71; VII, 68; VIII, 150; eyestalk short and stout conical, almost tuberculate; legs relatively large and long as compared to the size of the body, and to a similar relation in other species in the genus, otherwise characteristic for the genus, tarsal claw with 1 or, more frequently, 2 small denticles on the inner face, spines on legs rather slender; beak elongate conical, 1-segmented, sometimes with an indefinite indication of a joint visible near base; thoracic spiracles characteristic for the genus, external opening of each surrounded by a spine cluster including some smaller spines close to the opening, but without a distinctly developed spine collar; with 7 pairs of simple, tubular, abdominal spiracles; derm pores of the usual quadrilocular disk type only, these occurring both dorsally and ventrally but much more abundant ventrally within the area inclosed by the ovisac band, varying noticeably in chitinization and to some extent in size, the more heavily chitinized ones with a depth nearly as great as their diameter; derm with setae scattered over the surface both dorsally and ventrally, most numerous in loose clusters anterior to the genital opening ventrally; arrangement of the body spine clusters corresponding to the patches of secretion showing on perfect specimens, and about as shown in figure; ovisac band broad, made up of spines with some quadrilocular disk pores along the outer margin and others paralleling but not adjacent to the inner margin, area enclosed by this band without any transverse rows of spines, in this respect differing from all the other species of the genus; anal ring oval to elongate oval, the pore band on each half distinctly but not prominently angulate anteriorly and posteriorly on its inner margin and normally continuous with the opposite band at the anterior and posterior ends of the ring, this again differing from the condition among most of the species in the genus; with the usual 6 anal ring setae.

This species has been reported from or is represented in the collections examined from the following localities:

Algeria, Antigua, Barbados, Bermuda, Brazil, British Guiana, Canal Zone, Ceylon, China, Costa Rica, Cuba, Dominica, Ecuador, England (in greenhouses), France, Guatemala, Hawaii, Italy, Jamaica, Java, Madeira, Mauritius, Mexico, Montserrat, Panama, Peru, Porto Rico, Portuguese East Africa, Saint Kitts, Saint Lucia, Saint Thomas (Virgin Islands), Saint Vincent, San Thomé (Africa), Santo Domingo, Straits Settlements, United States (in greenhouses), including California, Connecticut, District of Columbia, Illinois, Indiana, Massachusetts, New Jersey, New York, Ohio, Pennsylvania, and Virginia.

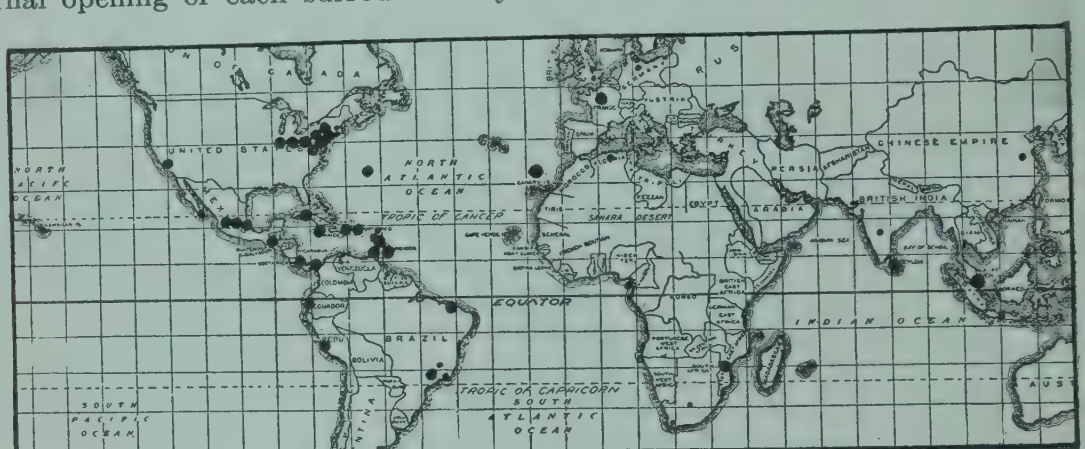


FIG. 18.—*Orthezia insignis:* Map showing actual known distribution. Large dots show records based on specimens actually examined; small dots show records based on published reports of occurrence

Host records on the same basis as distribution, that is, including both published records and those based on identifications of material, are as follows:

Abutilon (Malvaceae), Achillea (Compositae), Achyranthes (Amarantaceae), Ageratum (Compositae), Aloysia (Verbenaceae), Atalantia (Rutaceae), Barleria (Acanthaceae), Bignonia (Bignoniaceae), Capsicum (Solanaceae), Catalpa (Bignoniaceae), Cestrum (Solanaceae), Chionanthus (Oleaceae), Chrysanthemum (Compositae), Cineraria (Compositae),

Citrus (Ruraceae), Clerodendron (Verbenaceae), Clitoria (Leguminosae), Coffea (Rubiaceae), Coleus (Labiatae), Conoclinium (Compositae), Crossandra (Acanthaceae), Cuphea (Myrtaceae), Duranta (Verbenaceae), Eranthemum (Acanthaceae), Eupatorium (Compositae), Euphorbia (Euphorbiaceae), Fragaria (Rosaceae), Gardenia (Rubiaceae), Habrothamnus (Solanaceae), Ipomoea (Convolvulaceae), Iresine (Amarantaceae), Ixora (Rubiaceae), Jacaranda (Bignoniaceae), Justicia (Acanthaceae, Lantana (Verbenaceae), Libonia (Acanthaceae), Ligustrum (Oleaceae), Lycopersicum, (Solanaceae), Magnettia (Rubiaceae), Mentha (Labiatae), Meyeria (Acanthaceae), Mysotis (Boraginaceae), Oxalis (Geraniaceae), Pelargonium (Geraniaceae), Peristrophe (Acanthaceae), Pilea (Urticaceae), Rosa (Rosaceae), Rubiaceae, Saccharum (Gramineae), Salvia (Labiatae), Scutellaria (Labiatae), Solanum (Solanaceae), Stevia (Compositae), Strobilanthes (Acanthaceae), Theae (Ternstromiaceae), Thunbergia (Acanthaceae), Verbena (Verbenaceae), Veronia (Compositae), Viola (Violaceae).

ORTHEZIA LASIORUM COCKERELL

Figs. 3, K; 5, L; 7, H; and 19

REFERENCE.—Cockerell, 1901, Canad. Ent. 33: 209.

ADULT FEMALE.—No specimens available for description of external appearance, the following therefore copied from the original description "about 2 mm. long; pale orange; ovisac (in specimens seen) not very long; two very long median white caudal lamellæ, about two-thirds length of body, curving over ovisac, but not attached to it. Dorsum covered with waxy secretion, but it is so easily deciduous that I have never found an adult with it sufficiently in place to describe," body membranous except for a definite chitinous patch attached to each side of the anal ring as in *annae;* antennae normally 7-segmented, lengths of one in microns as follows: I, 107; II, 71; III, 107; IV, 71; V, 50; VI, 47; VII, 132; spine, 21; eyestalk conical, protruding, slightly curved; legs mutilated, structure uncertain; beak stout conical, 1-segmented, with an indistinct suggestion of a joint near base; thoracic spiracles set in a spine cluster and with a fairly distinct loose collar of spines around the opening of each; with 8 pairs of long tubular abdominal spiracles; derm pores of the quadrilocular disk type only, these occurring both dorsally and ventrally, usually in more or less distinct, but very scattered transverse rows, most abundant ventrally in the area inclosed by the ovisac band, fairly well chitinized, varying only slightly in size; body with slender setae occurring occasionally both dorsally and ventrally, most abundant, but not distinctly clustered, in the area inclosed by the ovisac band; derm spines arranged in the usual dorsal and lateral marginal clusters about as shown in figure, the dorsal bands larger and continuous to the marginal clusters; ovisac band broad, made up of spines, with an irregular single row of disk pores along the inner margin, and, anteriorly at least, an irregular double or triple row of similar pores along and

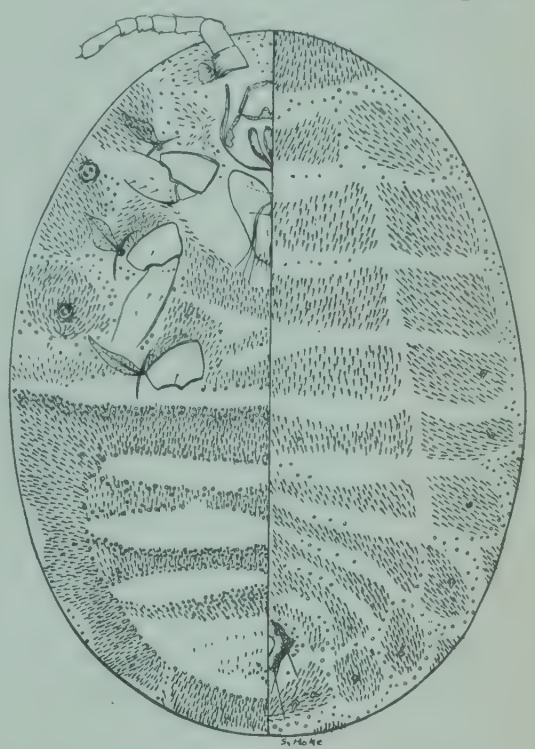


FIG. 19.—*Orthezia lasiorum:* Adult female, body, dorsal and ventral; × about 31

within the outer margin; ovisac band inclosing 3 broad, heavy, and 2 narrower, lighter bands of spines and pores, the first 3 of these extending broadly to the ovisac band on each side; anal ring elongate oval, heavily chitinized, with a relatively narrow pore band on each half, this made up of comparatively few pores and indefinitely angulate anteriorly and posteriorly, the 2 pore bands distinctly separated anteriorly and barely touching posteriorly, the middle of the outer face of each half of the anal ring with a protruding triangular chitinous wing, and just beyond this, on each side, 3 to 6 smaller, stout conical spines, this species differing, in the possession of the last, from all of the other species of the genus examined; anal ring with the usual 6 setae.

This species has been redescribed from the following material: A single mutilated specimen, mounted on a slide, from Las Vegas, N. Mex., in nests of *Lasius americanus*, collected by Mrs. T. D. A. Cockerell, April, 1901 (type).

ORTHEZIA LONGIPES HEMPEL

Figs. 3, L; 5, L; 7, H; and 20; Pl. 1, I

REFERENCE.—Hempel, 1920 (rec'd 1921), Rev. Mus. Paulista 12: 343, 345, 367–368.

ADULT FEMALE.—Very closely related to *O. praelonga* Dougl., differing clearly from that species, as usually recognized, only in the possession of a more elongate, asymmetrical conical eyestalk and of scattered spines just within the ovisac band anteriorly and posteriorly in addition to the normal 4 transverse rows or bands of spines.

FIG. 20.—*Orthezia longipes:* Adult female, body, dorsal and ventral; × about 31

The great kindness of the describer of the species, Mr. Hempel, in inducing the authorities of the Museo Paulista to send cotype specimens for examination has made it possible to compare this species with other members of the genus and to associate it in the relationship indicated.

The species is known only from the original collection at Rio de Janeiro, Brazil, on an undetermined native plant.

ORTHEZIA MEXICANA, NEW SPECIES

Figs. 3, M; 6, A; and 21; Pl. 1, J

ADULT FEMALE.—Stout oval, length of body with secretion about 1.8 millimeters, width about 1.1 millimeters, length of ovisac about 2.2 millimeters, completely covered dorsally with well-developed tufts of white secretionary matter, these arranged in the usual marginal and dorsal plates, the posterior marginal tufts increasing in length and projecting backwards over the ovisac; ovisac distinctly ribbed dorsally; length of body as mounted about 2.1 millimeters, width 1.7 millimeters, stout oval, tapering slightly anteriorly, posterior apex broadly rounded; derm membranous; antennae normally 8-segmented, the measurements of the segments of one in microns as follows: I, 132; II, 114; III, 94; IV, 93; V, 82; VI, 75; VII, 61; VIII, 158; spine, 36; eyestalk short, rounded conical, small; legs characteristic for the genus, rather short and stout, tarsal claw, at most, with a very indistinct denticle; setae on legs small, slender, spinelike, tarsal digitules relatively slender; beak elongate conical, 1-segmented, but with a quite distinct suggestion of a joint about one-third of length from base; thoracic spiracles characteristic for the genus, each opening into a spine cluster, and with spines grouped about this opening to form a very indefinite collar; with 7 pairs of large, short tubular abdominal spiracles; derm pores of the usual quadrilocular disk type, occurring rather commonly both dorsally and ventrally and very numerous in the ventral abdominal area inclosed by the ovisac band; derm with occasional setae both dorsally and ventrally, these most abundant in a well developed cluster anterior to the genital opening; body spines arranged in the usual 11 marginal and 10 dorsal clusters, about as shown in figure, the dorsal abdominal bands narrowed through much of their length, but broadened exteriorly, and all extending close to the marginal bands on each side; body spines, in general, rather elongate, slender, stout at base, tapering strongly near base, then gradually, to an almost pointed tip, but with the spines in the last pair of dorsal abdominal bands distinctly

shorter and smaller than those in the remainder; ventral ovisac band broad, made up of closely crowded spines, with bands of disk pores 3 or 4 deep extending through the exterior fourth or fifth of the band, but without any distinct pores along the inner margin, in this respect differing from most of the species in the genus, the concentration of disk pores usually occurring in or along the inner rather than the outer margin; ovisac band enclosing, besides the pores already mentioned, 5 more or less distinct transverse rows of small truncate conical tubercles, these occupying the position of and apparently corresponding to the rows of ventral abdominal spines found in nearly all the other species in the genus; anal ring stout oval, the pore band on each half broad, slightly joined at the ends, and distinctly and prominently angulate within anteriorly and posteriorly; with the usual 6 anal ring setae.

This species has been described from three mounted and a few unmounted adult females from Mexico (no definite locality) on roots of *Parthenium argentatum* (Compositae), collected by F. E. Lloyd, received February 5, 1909.

The types are in the United States National Collection of Coccidae.

FIG. 21.—*Orthezia mexicana:* Adult female, body, dorsal and ventral; × about 31

ORTHEZIA MINOR, NEW SPECIES

Figs. 3, N; 6, B; 7, I; and 22; Pl. 1, K

ADULT FEMALE.—Small, length of body with secretion about 2 millimeters, width about 1 millimeter, completely covered dorsally and ventrally with strongly developed plates of waxy secretion, buff-colored in the specimens examined, these arranged in the usual lateral and dorsal plates, the anterior

FIG. 22.—*Orthezia minor:* Adult female, body, dorsal and ventral; × about 31

median plate directed forward, long and deeply notched apically, the anterior lateral marginal plates very irregular, the posterior lateral plates increasing in length to the apical one or two, these quite long, anterior dorsal plates fused into a single transverse plate directed forward; the second pair separated, but also directed forward; remaining plates very strongly and conspicuously developed, directed diagonally backward and outward, overlapping for much of their length; ovisac short (possibly incompletely developed), only about 1 millimeter long; length of body, asmounted, 1.25 millimeters; width, 0.9 millimeter, ovoid, posterior apex broadly rounded, tapering anteriorly; derm membranous; antennae normally 8-segmented, rather elongate, measurements of one in microns as follows: I, 107; II, 100; III, 114; IV, 71; V, 78; VI, 75; VII, 76; VIII, 210; eyestalk rather elongate tubular; beak conical, 1-segmented, with a fairly distinct suggestion of a division near base; legs characteristic for the genus, relatively long as compared to the size of the body, rather slender, tarsal claw usually with 2 fairly distinct denticles on the inner face; thoracic spiracles not unusual, each opening into the usual spine cluster, with a few spines directly

encircling the opening but without a conspicuous spine collar around this, with, so far as can be determined, 4 pairs of long tubular abdominal spiracles; derm with the usual quadrilocular disk type of pores occurring both dorsally and ventrally, these all apparently rather lightly chitinized; with setae occasionally both dorsally and ventrally, these slightly but not conspicuously clustered anterior to the genital opening; with the usual 11 marginal and 10 dorsal clusters of spines, the latter broad, complete bands, each extending close to its corresponding marginal cluster on each side; ovisac band broad, interrupted posteriorly, made up of spines only,

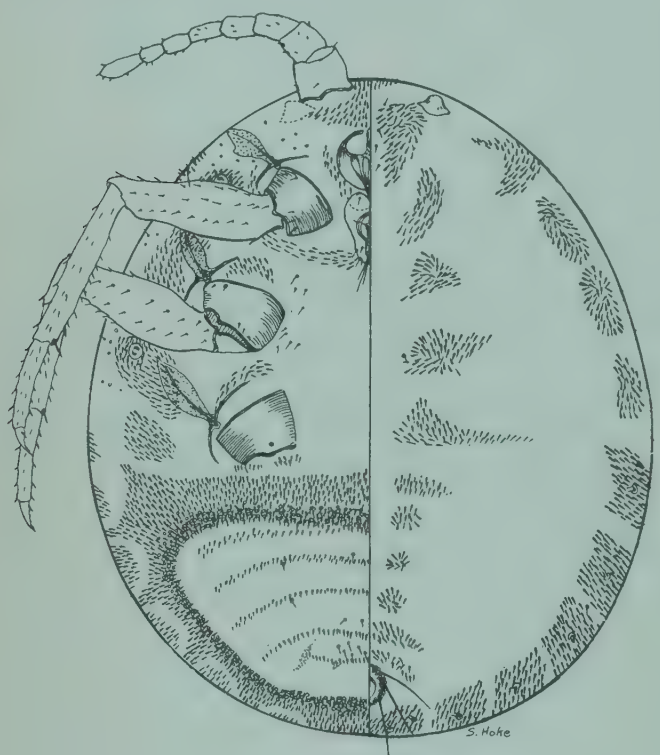


FIG. 23.—*Orthezia monticola:* Adult female, body, dorsal and ventral; × about 31

with a single row of scattered quadrilocular disk pores along the posterior margin, these somewhat larger and more heavily chitinized than the average for the remainder of the body; band inclosing 5 narrow, well-separated, loose transverse rows of spines, the anterior 3 extending close to the ovisac band on each side, the posterior 2 distinctly shorter; anal ring short oval, almost circular, pore band on each half divided into two parts posterior to the middle seta only, broad, plainly but not conspicuously angulate anteriorly and posteriorly, the ends of the two halves slightly but distinctly separated from each other; with the usual 6 anal ring setae.

This species has been described from two mounted and a very few unmounted specimens from Loona de Joaquin, Oriente Province, Cuba, on *Graffenriedia chrysandra* (Melanostomaceae), collected by C. H. Ballou and S. C. Bruner, July 20, 1922 (coll. No. 366).

The types are in the United States National Collection of Coccidae.

ORTHEZIA MONTICOLA COCKERELL

FIGS. 3, O; 6, C; and 23; Pl. 1, L

REFERENCE.—Cockerell, 1898, Ann. and Mag. Nat. Hist. (7) 2: 402.

ADULT FEMALE.—Dried body very broad oval, nearly circular, naked dorsally except for marginal and paired median tufts of secretion, in this respect superficially resembling *Orthezia insignis* to a marked degree, marginal tufts of secretion flattened, platelike, somewhat curved, the posterior ones much longer, sometimes approximating half the length of the body and distinctly separated for their entire length up to the anal tufts; ovisac variable in length, maximum apparently nearly 3 millimeters broad, distinctly ribbed dorsally; body color of dried specimens dark chestnut brown; length of body as mounted 2.1 millimeters, width 1.75 millimeters; derm membranous; antennae normally 8-segmented, length of segments of one in microns as follows: I, 114; II, 107; III, 125; IV, 103; V, 96; VI, 86; VII, 79; VIII, 143; spine, 15; eyestalk flat conical, apex rounded, legs characteristic for the genus, rather stout, tarsal claw fairly stout, with 2 or 3 indistinct denticles on the inner face; beak short and stout conical; thoracic spiracles characteristic for the genus, opening in a cluster of spines, but without distinctly developed spine collar; with 7 pairs of tubular abdominal spiracles; derm pores of the usual quadrilocular disk type, occurring both dorsally and ventrally, varying distinctly in extent of chitinization and size; derm with a few scattered setae both dorsally and ventrally, these more abundant in a loose cluster anterior to the genital opening; derm spines arranged in the usual dorsal and marginal clusters, widely separated, but with a slight tendency on the part of the dorsal clusters to form a transverse band; ovisac band fairly stout anteriorly, narrowed posteriorly, made up

numerous spines with a relatively rge number of disk pores scattered rough the inner one-third to one-half the band; area inclosed by the band ith 5 narrow bands of scattered spines xtending nearly to the ovisac band on ach side; anal ring elongate oval, ne pore band on each half rather arrow and not angulate within; anal ng with the usual 6 setae.

This species has been redescribed om the following material: Dripping prings, Organ Mts., N. Mex., on roots f grass (Gramineae), coll. T. D. A. ockerell (type material). The species oes not appear to have been observed nce its original discovery.

ORTHEZIA NIGROCINCTA COCKERELL

Figs. 3, P; 6, D; 7, J; and 24; Pl. 1, M

REFERENCE.—Cockerell, 1895, Amer. Nat. 29: 730.

Adult female.—Dried body nearly ircular, about 2.25 millimeters long y 2 millimeters wide; with the usual marginal tufts of secretion, these all airly long, mostly curved backward nd flattened over the ovisac, and with he usual series of dorsal tufts, but these listinctly separated from the body nargin by a bared blackish band on ach half, this band, however, narrower han in either *insignis* or *monticola*, the ombination of dorsal tufts occupying ully two-thirds of the disk of the lorsum, tufts narrow and erect anteiorly, broader and considerably wider hrough the thoracic segments and gain narrower, except for the last pair, n the abdominal region, closely approximated along the median line, with nly a linear bare streak more or less efinitely exposed; ovisac attaining a ength greater than that of the ody, distinctly ribbed dorsally, slightly urved upward and slightly tapering osteriorly; body of female as mounted bout 2 millimeters long by 1.5 millimeters wide, oval, tapering somewhat nteriorly; derm membranous, except or an elongated, more or less definitely efined, chitinized plate running back orsally from the anterior margin of he head; antennae normally 8-segmented, measurements of one in icrons as follows: I, 125; II, 100; III, 40; IV, 125; V, 115; VI, 100; VII, 103; VIII, broken; eyestalk stout conical, pex rounded, heavily chitinized; legs ot unusual for the genus, fairly stout, arsal claw with 2 or 3 indistinct denicles on the inner face; beak fairly ng conical, 1-segmented, with a suggestion of a joint sometimes visible ear base; thoracic spiracles entirely haracteristic for the genus, the opening surrounded by an indefinite collar of smaller, stouter spines; with 8 pairs of short tubular abdominal spiracles; derm with the usual quadrilocular disk pores, both dorsally and ventrally, these varying conspicuously in size, most abundant in the ventral area inclosed by the ovisac band; derm with occasional setae dorsally and ventrally, these most abundant in a definite cluster just anterior to the genital opening; body spines arranged in the usual 11 marginal and 10 dorsal clusters on each half, about as shown in figure, the dorsal clusters, while distinctly and mostly widely separated from the corresponding marginal clusters, at the same time plainly transverse; ventral ovisac band rather stout, composed of numerous spines with an irregular row of disk pores 2 or 3 deep along the inner margin; ovisac band enclosing 3 short, transverse, narrow rows of spines, none of these extending to or even close to the ovisac band at the sides; anal ring elongate oval, the pores on each half united or nearly so anteriorly and posteriorly, and the inner margin of each pore band fairly distinctly angulate anteriorly and posteriorly; ring with the usual 6 setae.

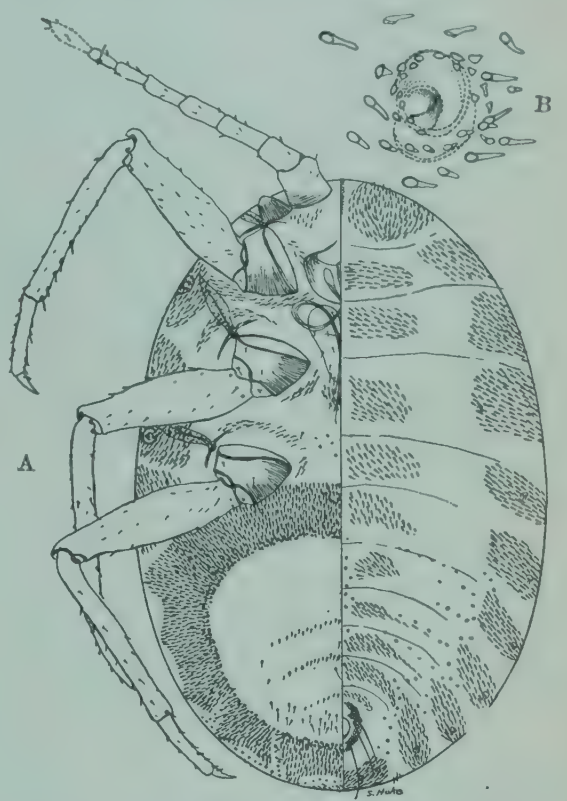


FIG. 24.—*Orthezia nigrocincta*, adult female: A, body, dorsal and ventral, × about 31; B, opening of thoracic spiracle, showing greatly reduced spines at edge of opening, × 230

The following material has been examined in connection with the redescription of this species: Mescalero Agency, N. Mex., on Gutierrezia (Com-

positae), coll. C. H. T. Townsend, Oct., 1896 (Div. Ent. No. 7288 (type); Beulah, N. Mex., on Artemisia (Compositae), coll. T. D. A. Cockerell, Aug., 1899; Las Valles, N. Mex., coll. T. D. A. Cockerell, Aug., 1899; Las Valles, N. Mex., coll. T. D. A. Cockerell, received July, 1907.

The published records add Embudo, N. Mex., to the distribution given in the preceding paragraph, but no other hosts.

ORTHEZIA NUDA FERRIS

Figs. 3, Q; 6, E; 7, K; and 25; Pl. 2, A

REFERENCE.—Ferris, Contr. Knowl. Cocc. S. W. U. S. Stanf. Univ. Pub. Univ. Ser., 1919, p. 13.

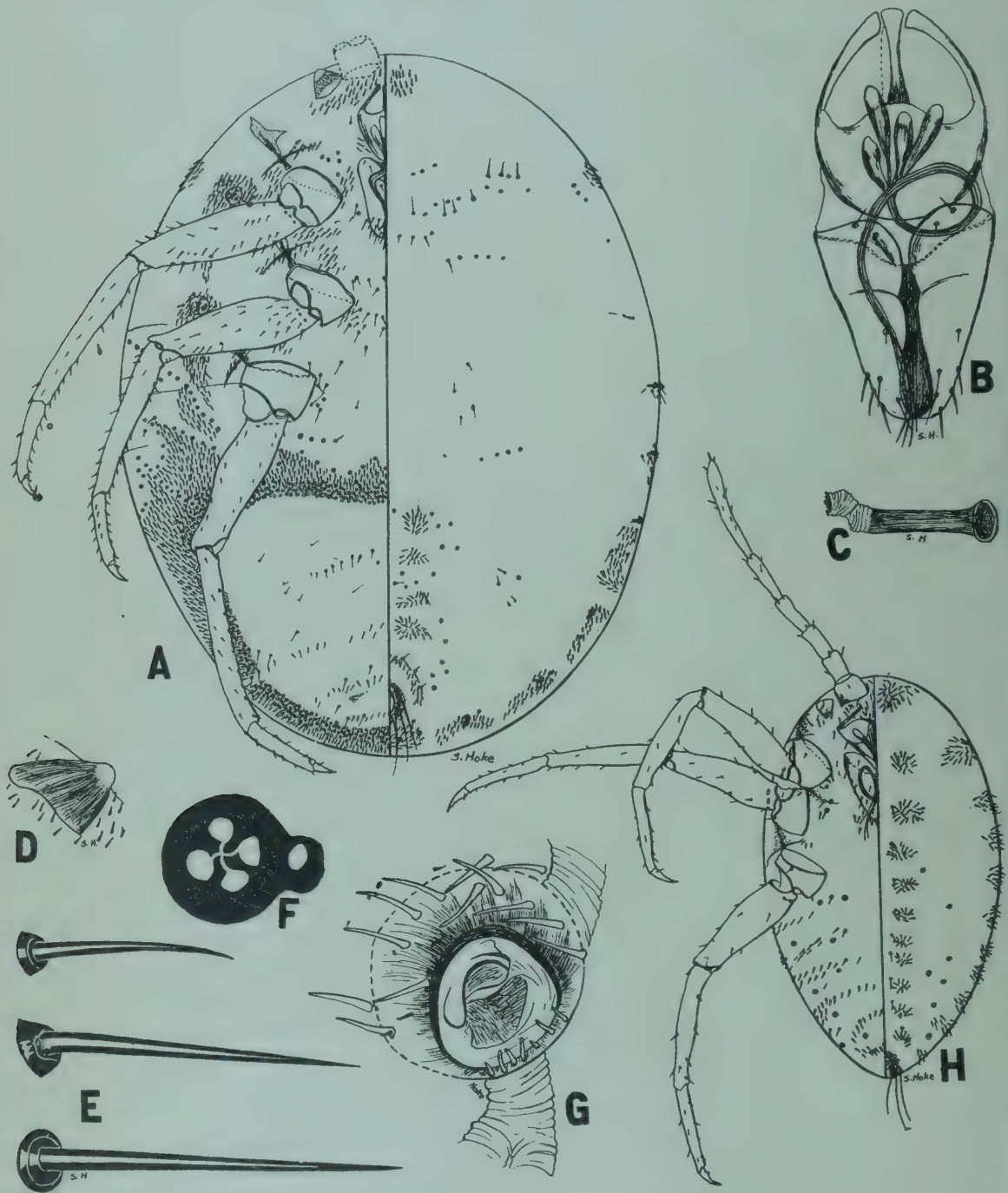
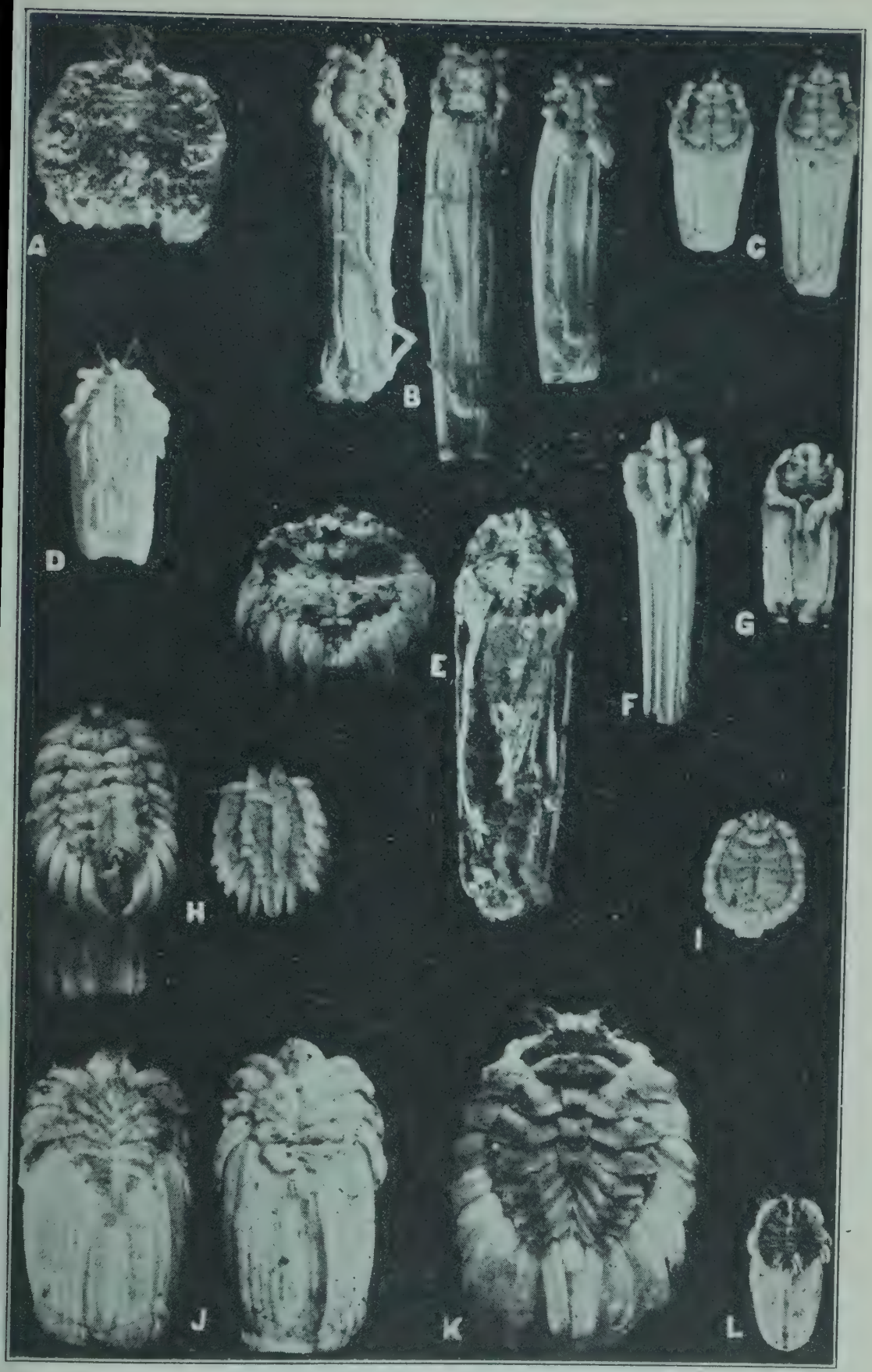


FIG. 25.—*Orthezia nuda:* A, adult female, body, dorsal and ventral, × 23; B, same, beak, × 60; C, same, eyestalk, × 60; D, same, abdominal spiracle, × 230; E, same, body setae, × 650; F, same, quadrilocular disk pore, with simple pore adjacent, × 1,500; G, same, thoracic spiracle, × 230; H, larva, body, dorsal and ventral, × 60.

ADULT FEMALE.—Dried and somewhat shriveled female dark reddish brown, margin lighter, translucent, length of body with ovisac 4 milimeters, width of body 2.5 milimeters, body dorsally destitute of secretion except



Scale Insects of the Subfamily Ortheziinae Plate 2

A, *Orthezia nuda*, adult female, ovisac wanting; B, *O. praelonga*, adult females, secretion mutilated; C, *O. pseudograminis*, adult females (photo by J. G. Sanders); D, *O. solidaginis*, adult female (photo by J. G. Sanders); E, *O. sonorensis*, adult females, secretion mutilated; F, *O. tillandsiae*, adult female; G, *O. ultima*, adult female; H, *O. urticae*, adult female and immature female; I, *O. cataphracta*, adult female, ovisac wanting; J. *Newsteadia americana*, adult females; K, *Orthezia occidentalis*, adult female, ovisac obscured; L, *Nipponorthezia ardisiae*, adult female, secretion incomplete

for 2 small tufts on the head between antennae and, normally, 5 indistinctly separated, small, median, paired tufts on the abdomen just anterior to the anal ring; anterior lateral margins of body likewise destitute of secretion, caudal margin with 3 tufts on each side of the middle line, and a fourth, smaller, just outside the third, but placed somewhat ventrally instead of at the margin; ovisac, in the specimens examined, short, very little longer than broad, with 8 more or less distinct dorsal ribs and fine longitudinal striations ventrally; body, as mounted on slide, stout oval, nearly as wide as long, length 3 millimeters, width slightly less; derm membranous except for a pair of elongate triangular transverse thickenings opposite genital opening ventrally; antennae normally 8-segmented, apparently not unusual; legs characteristic for the genus, tarsal claw rather stout, claw digitules stout, spinelike, inner face of claw with 3 denticles; eyestalk conical, apex rounded, with a more or less developed basal chitinous lip; beak of medium size, short conical, apex rounded, 1-segmented, but with a fairly distinct suggestion of a joint about one-third of its length from the base, apex bearing several stout setae; thoracic spiracles characteristic for the genus, the openings of each surrounded by a cluster of spines, with a few of these shorter and otherwise modified from the typical forms; with 8 pairs of elongate tubular abdominal spiracles; derm pores of the quadrilocular disk type only, but of two sorts, one, the larger, heavily chitinized, with relatively large loculi and relatively small marginal rim, these occurring mostly scattered over the dorsal surface but occurring also, somewhat reduced in size and distinctly reduced in chitinization, in connection with the ovisac band and elsewhere in the ventral region, the other, much more faintly chitinized and with relatively much smaller loculi and larger marginal rim, occurring chiefly in the midventral abdominal region within the area surrounded by the ovisac band, many of these, particularly among the larger dorsal pores, with one or more short lateral chitinous extensions, each running to and surrounding a small, simple, clear circle or with such small circles close to but separated from the disk pore; these disk pores scattered, except along the inner margin of the ovisac band, here mostly closely crowded and extending perhaps one-fourth of its width through the band; body with rather stout setae, apparently indiscriminately scattered over it, or rarely, as in the midventral region, in indistinct transverse rows, and with 1 transverse cluster anterior to the genital opening; spines present dorsally in a paired transverse cluster just anterior to the anal ring and in 4 pairs of small irregular median clusters on the abdominal segments just anterior to this, these spines stout, normally tapering only very slightly after the initial constriction at the bases, and with their apices bluntly rounded; with a small, probably paired, marginal tuft at the anterior apex of the body; a small marginal tuft opposite each anterior spiracle and undoubtedly with small marginal clusters responsible for production of the marginal abdominal tufts of secretion along the apex and sides of this, but these so mutilated in the few specimens available for study that little can be said regarding their character; ovisac band rather narrow, composed of spines similar to, but more slender and less heavily chitinized than, those found dorsally, accompanied by a band of pores several deep along the inner margin and extending into the spine band for some distance, inclosing at least 3 transverse rows of spines; anal ring characteristic for the genus except that the inner margins of the pore band are not angularly produced near the anterior and posterior ends; with the usual 6 anal ring setae.

LARVA.—Oval, somewhat tapering behind, short, length about 715 microns; width about 430 microns; antennae 6-segmented, the terminal fully twice the length of any of the others; eyestalk conical; legs not unusual, claw slender, with 2 distinct denticles and well developed, spinelike digitules; beak 1-segmented; thoracic spiracles not unusual; abdominal spiracles elongate, slender, with 8 pairs as in adult; derm spines tapering; with occasional quadrilocular disk pores, these in more or less distinct longitudinal rows; ventrally bearing transverse submarginal segmental rows of elongate, rather slender spines in the abdominal region, these clusters more irregular in shape than in the thoracic region, and dorsally with small, submedian clusters of similar spines the whole length of the body and somewhat similar marginal tufts corresponding segmentally with the median; anal ring not unusual.

Owing to the discovery of 8 pairs of abdominal spiracles and of small clusters of spines on the dorsum of the abdomen, the specimens available for study were originally described as a new species, but through the great kindness of Professor Ferris in loaning the single mounted specimen of his

pecies, it has been possible to ascer- ain that these specimens are actually dentical with his species, although his lescription should be corrected with espect to these two points in its tructure. In having the dorsal spine lusters that are present of very small ize and in two longitudinal rows on he abdomen, the species resembles *nonticola* and *insignis*, from which it an be definitely separated, not only y the incompleteness of the dorsal pine rows and secretion, but by the resence of 8 pairs of abdominal spira- les.

This species has been redescribed rom two mounted adult females, ne unmounted female, and several nounted larvae collected by H. S. Barber and Dr. E. A. Schwarz in the Santa Rita Mountains, Ariz., June 14, 1905, on an unknown host. The type specimens of the species were collected near Benson, Ariz., on *Quercus emoryi* (Fagaceae) by Prof. Ferris, June, 1918.

ORTHEZIA OLIVACEA COCKERELL

Figs. 3, R; 6, F; 7, L; and 26

REFERENCE.—Cockerell, 1905, Canad. Ent. 37: 136.

ADULT FEMALE.—No specimens available for description of external appearance, the following, therefore, copied from the original description: 'Length about 2.5 mm., with cauda rather over 3 mm., legs and antennæ reddish-brown. Body entirely covered with dense white secretion; dorsal line marked by a deep groove, with no median tufts; the two dorsal rows of lamellæ thick and obtuse, the first pair overlapping head, but not projecting far forward; area between dorsal and lateral lamellæ covered by secretion; lateral lamellæ broad, the anterior three truncate, the others more pointed, the points curved inward; caudal lamellæ surpassing last lateral ones, but not very long. Body denuded of lamellæ dark olivaceous.'' Length of body as mounted about 2.25 millimeters, width 1.75 millimeters, uniformly oval; derm membranous; antennae normally 7-segmented, lengths of one in microns as follows: I, 96; II, 89; III, 93; IV, 50; V, 50; VI, 46; VII, 139; spine, 21; eyestalk very small, flattened, the eyespot occupying most of the tuberculate center; legs rather small, fairly stout; setae slender, tarsal claw with 2 or 3 fairly distinct denticles on the inner face; beak stout conical, 1-segmented, but with a rather distinct suggestion of a joint near the base; thoracic spiracles characteristic for the genus, each opening within a spine cluster and this opening surrounded by a rather definite collar of somewhat smaller and shorter spines; with 8 pairs of long tubular abdominal spiracles; with the usual quadrilocular disk type of pores only, these occurring both dorsally and ventrally, mostly not heavily chitinized, very abundant in the posterior portion of the area inclosed by the ovisac band; derm with a few scattered setae both dorsally and ventrally; derm spines arranged in the usual 11 marginal and 10 dorsal clusters, about as shown in figure, the dorsal bands fully developed and extending almost to the corresponding marginal bands on each side; ovisac band stout, made up of spines with disk pores along the anterior margin, with a scattered row of distinctly smaller disk pores just within the inner margin of the band; inclosing 5 transverse bands of spines, the 3 anterior of these broad and extending fully to the ovisac band on each side; anal ring stout oval, the pores on each half produced within anteriorly and posteriorly but not angularly, meeting anteriorly but slightly separated behind; ring with the usual 6 rather long setae; with tiny hemispherical tubercles scattered through the areas between the spine clusters on each side of the anal ring, both dorsally and ventrally.

FIG. 26.—*Orthezia olivacea:* Adult female, body, dorsal and ventral; × about 31.

This species has been redescribed from the following material: From

Boulder, Colo., in nests of *Lasius* sp. under rocks, coll. W. P. and T. D. A. Cockerell, November, 1904 (type), and Colo. with ants, coll. C. F. Baker, about 1897 (from Tinsley collection).

The species was also recorded in the original description as occurring at Trout Spring, N. Mex.

ORTHEZIA PRAELONGA DOUGLAS

Figs. 3, S, T; 6, G; 7, M; 27 and 28; Pl. 2, B

REFERENCE.—Douglas, 1891, Ent. Mo. Mag. 27: 246–247.

ADULT FEMALE.—Length of adult female with secretion nearly 2 millimeters, width 1.25 millimeters, ovisac variable in length, sometimes as much as 6 millimeters long, more or less distinctly ribbed dorsally, body completely covered with very fragile white secretion dorsally, showing a more or less distinct, but at most narrow, bare streak near each margin, separating the dorsal and marginal plates, secretion evidently arranged in the usual lateral and dorsal tufts, but invariably so confused medially, in museum specimens, as to make its accurate description practically impossible; the 2 anterior marginal plates elongate conical, protruding directly forward, the next lateral marginal plates also elongate, protruding directly laterad, the remaining marginal plates more and more strongly curved backward, with the plates of the apical pairs quite long, projecting over the ovisac; the 2 anterior pairs of dorsal plates directed forward, the next nearly erect, and the remainder directed somewhat backward and overlapping; ventral surface completely covered with secretion except at the insertions of the appendages; body of female, as mounted, about 1.5 millimeters long by 1 to 1.25 millimeters wide, rarely somewhat larger than this; uniformly oval, or somewhat narrowed anteriorly; derm membranous, except for a narrow, dorsal, chitinized, median stripe running backward from a point at or close to the anterior margin, varying somewhat in length and width, and frequently, but not always, expanded at or near the posterior apex; antennae normally 8-segmented, but often abnormal, as few as 5 segments having been observed, lengths of segments of one in microns as follows: I, 118; II, 93; III, 157; IV, 124; V, 118; VI, 107; VII, 114; VIII, 221; spine, 22; eyestalk rounded tuberculate, somewhat conical, small; legs quite long in proportion to the size of the body, in this respect resembling *insignis*, moderately slender, bearing slender setae, tarsal claw with 2 distinct, but not prominent, denticles on the inner face, more rarely with a slight swelling suggesting the presence of a third basad to the other 2; beak conical, apex rounded, 1-segmented, with a very faint suggestion of a division line near base occasionally showing; thoracic spiracles entirely characterictic for the genus, opening in a spine cluster and with some spines grouped about the opening in the form of an indistinct spine collar; with 7 pairs of long tubular abdominal spiracles; derm pores of the usual quadrilocular disk type, occurring both dorsally and ventrally, varying somewhat in size and chitinization, most abundant along the margin of the ovisac band, but also fairly numerous in the posterior ventral abdominal area; derm with scattered slender setae both dorsally and ventrally, in addition with some small spinelike setae just anterior to the genital opening, and with a considerable number of small circular clear disks with chitinized rims in a loose cluster posterior to the same, and to a less extent anteriorly among the clusters of small setae; derm spines arranged in the usual 11 marginal and 10 dorsal clusters about as shown in figure, the dorsal abdominal bands rather narrow, with wide interspaces, and, while continuous to the marginal bands on each side, somewhat more widely separated from the last than in many other species, the spines composing them more loosely and less definitely grouped than in many of the other species; ovisac band broad, made up of spines, with the addition of a large number of disk pores, in a row 4 to 6 deep clear around the inner margin of the band and partly confused with the

FIG. 27.—*Orthezia praelonga:* Adult female, body, dorsal and ventral; × about 31

spines along this margin and, along the anterior margin of the band, in a similar pore row, averaging about 3 to 5 deep, and definitely intermingled with the spines making up the band along this margin; ovisac band interrupted laterally and posteriorly, inclosing 4 distinctly separated transverse bands of scattered spines, these extending to the ovisac band on each side, the anterior 2 about 3 or 4 spines wide, the posterior 2 narrower, the last composed mostly of only a single row of spines; anal ring oval, characteristic for the genus, the pore bands on each half slightly separated at their ends and the inner margin of the same distinctly and acutely angulate anteriorly and posteriorly; with the usual 6 anal ring setae, these rather long and slender.

FIG. 28.—*Orthezia praelonga:* Map showing actual known distribution. Large dots show records based on specimens actually examined; small dots show records based on published reports of occurrence.

Material from the following localities has been more or less carefully examined during the preparation of the preceding description: Barbados, Bolivia, Brazil, British Guiana, Canal Zone, Ecuador, Grenada, Panama, St. Croix (Virgin Islands), and Trinidad. Published distribution records add Antigua, Dominica, Jamaica, and St. Kitts to the above list.

The host records, both for material examined and from published information, include: Capsicum (Solanaceae), Carica (Passiflorae), Citrus (Rutaceae), Cocoloba (Polygonaceae), Codiaeum (Euphorbiaceae), Coffea (Rubiaceae), Euphorbia (Euphorbiaceae), Gossypium (Malaceae), Haematoxylon (Leguminosae), Hyptis (Labiatae), Lonicera (Caprifoliaceae), Loranthus (Loranthaceae), Malphigia (Malphigiaceae), Parderia (Rubiaceae), Rosa (Rosaceae), Saccharum (Gramineae), Sanchezia (Acanthaceae), and Sapium (Euphorbiaceae).

ORTHEZIA PSEUDOGRAMINIS, NEW SPECIES

Figs. 3, U, and 29; Pl. 2, C

ADULT FEMALE.—External appearance in general resembling that of *graminis* very closely, differing principally in the following details: Dorsal bare strip on each half of the body normally extending only over about the anterior half of the body length, the remainder of this area covered with secretion, although not with prominent erect plates; marginal fingerlike tufts of secretion along the posterior portion of the body somewhat longer than in *graminis;* ovisac averaging not more than two-thirds of the maximum length of that of *graminis* in specimens examined; shape of body, as mounted, much as in *graminis,* size averaging slightly smaller; relative lengths of antennal segments approximately the same; legs somewhat stouter than in *graminis;* tarsal claw with denticles; dorsal and marginal spine clusters in general corresponding to those of *graminis,* but with the dorsal abdominal clusters forming complete bands extending to the corresponding marginal clusters and separated from the same only by a narrow clear area, and with the two posterior dorsal thoracic clusters united with the corresponding marginal clusters by a very narrow band of scattered spines.

FIG. 29.—*Orthezia pseudograminis:* Adult female, dorsal and ventral; × about 31

This species has been described from the following lots of material: Sterling, Colo., on *Sporobolus cryptandrus* (Gramineae), coll. E. O. G. Kelly, July, 1908 (type and paratypes); Lamar, Colo., on *Deschampsia cespitosa* (Gramineae), coll. E. D. Ball, Sept., 1898 (paratypes); Pecos, Tex., on salt grass (*Distichlis spicata*) (Gramineae), coll. C. N. Ainslie, July, 1908 (paratypes); Mesilla Park, N. Mex., on grass (Gramineae), Oct. 19, 1897, coll. Cockerell (paratypes).

The types are in the United States National Collection of Coccidae.

FIG. 30.—*Orthezia solidaginis:* Adult female, dorsal and ventral; × about 31

The species described above is extremely close to typical *graminis*, differing definitely only with respect to the extent of the lateral development of the dorsal spine bands as described and illustrated, and it has, in consequence, been very reluctantly characterized as new, since it seems not entirely unreasonable that these dorsal bands might vary to this extent. The material at hand, however, where in a condition for accurate study, fails to disclose any marked variation in series of specimens from any one lot, or anything approximating a definite range of intergradation between the conditions of the spine development which appear to delimit the two species. The apparent occurrence of both species at Mesilla Park is evidence in favor of the assumption that the difference in the extent of the lateral development of dorsal spine bands is merely due to variation, but such an assumption needs verification by some positive evidence of variation or intergradation before it can be fully accepted and the species described herewith as new be reduced to synonymy. This description will at least serve to emphasize the need for an examination of a long series of specimens of these grass-inhabiting forms.

ORTHEZIA SOLIDAGINIS SANDERS

Figs. 3, V; 6, H; and 30; Pl. 2, D

REFERENCE.—Sanders, 1904, Ohio Nat. 4: 94–95, pl. 8, figs. 57–63.

SYNONYM.—*Orthezia ambrosiae* Lawson, 1917, Univ. Kan. Bul. Biol. Ser. 18: 165–167, fig. 1.

ADULT FEMALE.—External appearance described in detail by Sanders in his original description, and by Lawson under the name *ambrosiae*, and illustrated in photograph accompanying this paper. Length of body, with ovisac, 6 to 7.5 millimeters; width about 2.5 millimeters; length of body, as mounted, about 2.75 millimeters; width about 2 millimeters, oval, tapering more or less distinctly anteriorly; derm membranous; antennae normally 8-segmented, measurements of one in microns as follows: I, 160; II, 143; III, 246; IV, 164; V, 171; VI, 150; VII, 133; VIII, 200; spine, 21; eyestalk rather strongly protruding, conical tuberculate; legs characteristic for the genus, rather long and slender, tarsal claw usually with 3 distinct denticles on the inner face; beak conical, 1-segmented, with a fairly distinct indication of a division line about one-third of the length from base; thoracic spiracles characteristic for the genus, the opening of each surrounded by a distinct and rather conspicuous, closely set collar of spines, a portion of this collar resting on a somewhat chitinized curved plate following the outline of the opening; with 8 pairs of long tubular abdominal spiracles; with the usual quadrilocular disk pores, these present both dorsally and ventrally, more numerous in and along the inner margin of the ovisac band and also rather abundant through-

out the area inclosed by this band; derm with an occasional seta both dorsally and ventrally; body spines arranged in the usual 11 marginal and 10 dorsal clusters, about as shown in figure, the dorsal clusters extending close to the marginal, with the outer end of dorsal cluster and the inner end of the marginal cluster sinuous so that the two overlap each other more or less obviously; ovisac band broad, interrupted posteriorly by 3 transverse clear bands on each side, made up of numerous crowded spines, and, through the inner third particularly, numerous and rather crowded disk pores, anterior margin with a more or less definite single row of these pores; ovisac band inclosing 5 transverse rows of spines, these distinctly and rather widely separated from one another, but, excepting the last, extending to the ovisac band at each end and more or less distinctly broadened at the ends; anal ring elongate oval, the pores on each nearly contiguous at the ends, although not united, the inner margins acutely angulate anteriorly and posteriorly; with the usual 6 fairly long setae, and with a few tiny clear disks on each side of the anal ring and more of these ventrally behind the anal ring, these apparently similar to those already described for *praelonga* and *olivacea*.

The preceding redescription of this species has been based on the type specimens from Columbus and Sandusky, Ohio, on *Solidago canadensis* (Compositae), coll. J. G. Sanders, Oct., 1902, and July, 1903, and on specimens from St. Louis, Mo., on Solidago, coll. Otto Lugger, July, 1872 (No. 234 L). Material from Plummers Island, Md., on Solidago, coll. J. G. Sanders, Oct., 1908, and from Round Hill, Va., on Potentilla (Rosaceae), coll. J. G. Sanders, Sept., 1909, has also been available. The published records add Kansas to the distribution and *Ambrosia trifida* (Compositae) to the known hosts.[5] The synonymy indicated for *ambrosiae* Lawson has been based on an examination of specimens received from Prof. Lawson by the Bureau of Entomology.

ORTHEZIA SONORENSIS COCKERELL

Figs. 3, W; 6, I; and 31; Pl. 2, E

REFERENCE.—Cockerell, 1896, U. S. Dept. Agr. Div. Ent. Tech. Ser. 4: 38–39.

ADULT FEMALE.—Very closely resembling *Orthezia annae;* antennae normally 8-segmented, measurements of one in microns as follows: I, 107; II, 126; III, 150; IV, 100; V, 103; VI, 89; VII, 85; VIII, 160; disk pores along inner margin of ovisac band somewhat less numerous than in *annae*, fewer of the inner spines of the posterior dorsal clusters reduced in size than in *annae*.

LARVA.—In general resembling that of *annae* quite closely, but differing conspicuously in that the antennae of all the specimens examined are only 5-segmented, with the third segment very long, as compared with the normal 6 segments present in the larva of *annae*, this difference apparently furnishing the only positive means of differentiating these two species.

FIG. 31—*Orthezia sonorensis:* Adult female, body, dorsal and ventral; × about 31

This species has been included in this paper on the basis of material from San Ignacio, Sonora, Mexico, on *Hymenoclea monogyra* (Compositae), coll. C. H. T. Townsend, Sept. 26, 1894 (type). It does not appear to have been observed since the publication of the original description.

[5] See also *Orthezia americana*, as most of the records for that species probably actually apply to *O. solidaginis*

ORTHEZIA TILLANDSIAE, NEW SPECIES

Figs. 4, A; 6, J; 7, N; and 32; Pl. 2, F

ADULT FEMALE.—Size small, average length of body with secretion 1.5 millimeters, average width of body with secretion nearly as much; ovisac when fully developed very elongate, total maximum length of ovisac and body observed 9 millimeters, average length approximately 6 millimeters, ovisac widest at base, tapering slightly posteriorly, more or less curved in a horizontal plane when fully developed, about as wide as the body of the insect and distinctly narrower than the body on each side, by a similar line isolating the median tufts of secretion from the marginal tufts; each margin of body with fingerlike tufts of secretion, the anterior curving forward, placed opposite the base of the anterior median tuft, the remainder curving backward with gradually increasing emphasis, until the posterior 2 are reached, these longer and more slender than the others; ovisac definitely, but not prominently, ribbed or fluted dorsally and laterally, only finely striated ventrally; body ventrally nearly as completely covered with secretion as the dorsal surface but the secretion

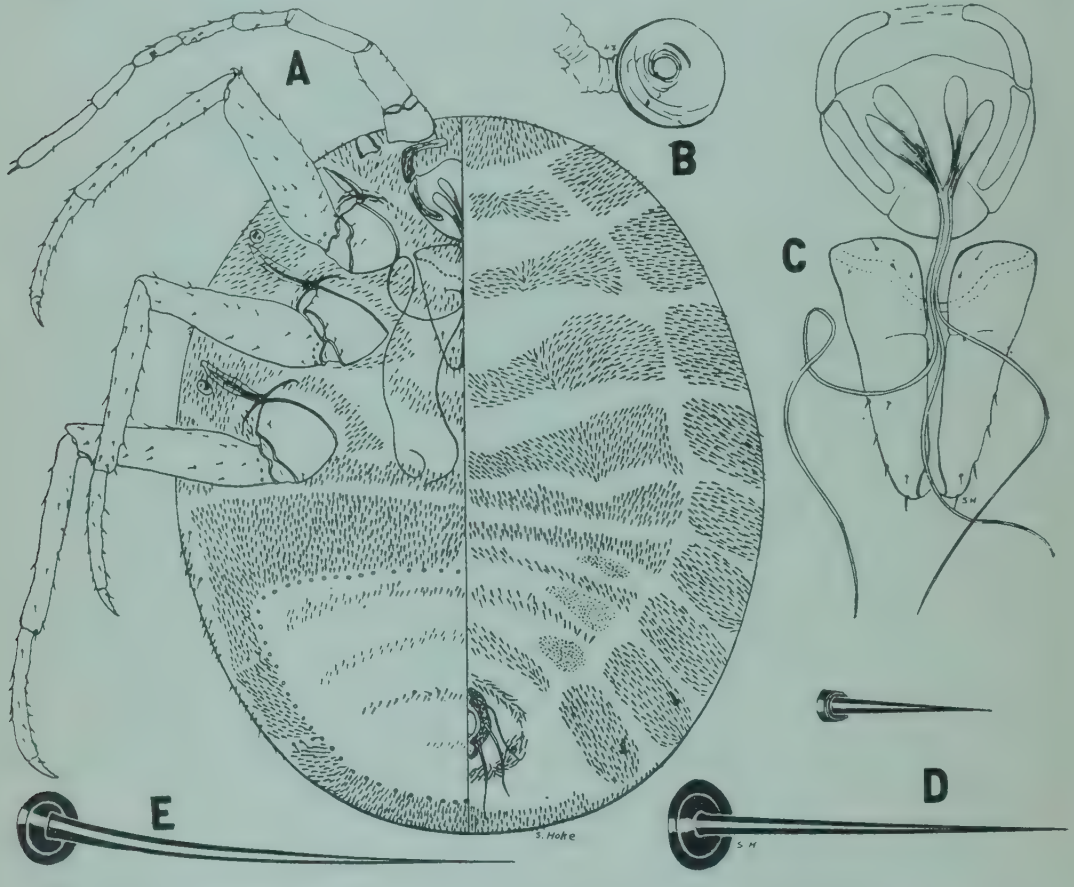


FIG. 32.—*Orthezia tillandsiae*, adult female; A, body, dorsal and ventral, × about 60; B, thoracic spiracle, × 335; C, mouth parts, × 120; D and E, body setae; × 1,500

with its secretion; body of female covered with firm waxy secretion divided into plates, with 2 very long anterior plates projecting well out beyond the head, 2 much smaller, transverse, just behind these, 3 pairs of semierect, posteriorly directed plates covering the disk of the thoracic region, and, in direct continuation of these, 6 similar plates gradually reduced in size posteriorly and usually so fused together that the actual number present is not determinable, these 2 rows of plates distinctly separated from each other by an impressed median line penetrating to the derm, and laterally, here much thinner and less prominent; average length of body as mounted on slide 1.3 millimeters, average width 0.9 millimeter; derm membranous except for 3 pairs of, usually, quite distinct, transverse, irregular to oval, somewhat chitinized areas placed dorsally on each side of the outer ends of, and between, the second to fifth transverse rows of spines anterior to the anal ring; antennae normally 8-segmented, the lengths of these for one antenna in microns as follows: I, 107; II, 89; III, 114; IV, 64; V, 93; VI, 75; VII, 79; VIII, 160; spine, 18; eyestalk nearly as long as wide, only slightly conical,

apex rounded, average length about 43 microns, width at base 50 microns; legs characteristic for the genus, tarsal claw nearly straight, fairly stout, with 2 distinct and sometimes 1 indistinct denticle within, claw digitules relatively slender; beak not unusual, length of that of an average specimen 196 microns, width at base 175 microns; 1-segmented, with only a faint fold about one-third of length from base suggesting a joint; thoracic spiracles not unusual, with a cluster of spines of normal shape and size around the opening of each; abdominal spiracles short and relatively stout, very obscure, owing to the close crowding of the body spines; exact number not absolutely determinable, but apparently with 4 pairs, these the 4 posterior ones; derm pores, so far as observed, of the one characteristic quadrilocular disk type only, occurring sparingly, except in the midventral abdominal region, here in rather closely set, irregular, transverse rows, particularly around the genital opening, and these last pores somewhat larger and less heavily chitinized and with a wider marginal rim than those occurring dorsally and laterally; body with scattered setae dorsally and laterally, in more or less distinct relation to the spine clusters, and with a distinct transverse cluster of setae of varying sizes just anterior to the genital opening, as well as with indistinct transverse rows of such setae anterior to this cluster; body spines distinctly set off in groups corresponding to the secretion as already described, the disk of the dorsum with 10 paired transverse clusters on the head, thorax, and abdomen, of which the first 4 are distinctly larger and broader than those on the abdomen; marginal spines, including the anterior head clusters and the posterior abdominal one, in 11 clusters on each side of the body, these placed more or less directly opposite the ends of the corresponding transverse dorsal clusters; anterior section of ovisac band relatively broad, accompanied only by a single row of scattered quadrilocular disk pores, lateral and apical sections of this band somewhat narrower, likewise accompanied only by the single row of pores, this band interrupted laterally by narrow, diagonal clear areas, and inclosing, in the midventral abdominal region, 3 narrow, curved, widely separated, transverse rows of short spines; with clusters of spines around the openings of the thoracic spiracles, but none of these unusual in shape; also with clusters and patches of spines around the coxae; anal ring of normal form for the genus, the inner margin of the pore band on each half strongly angulate within anteriorly and posteriorly, the angulate tongues nearly inclosing a membranous area at each end of the ring; with the usual 6 anal ring setae.

LARVA.—Ovate, tapering somewhat behind, average length 464 microns, average width 303 microns, eyestalk short tuberculate, antennae not unusual, lengths of segments of one in microns as follows: I, 32; II, 39; III, 43; IV, 50; V, 50; VI, 121; spine, 21; legs normal, fairly stout, tarsal claw with 2 prominent denticles on the apical half, and frequently with a somewhat less conspicuous one about the middle, claw digitules set alike, and not over one-fourth the length of the claw; beak elongate conical, average length about 93 microns, average width at base about 71 microns; thoracic spiracles not unusual; abdominal spiracles apparently in 4 pairs as in adult; with a few scattered derm pores, these forming short, indefinite, median transverse segmental rows dorsally in the thoracic region; body with a few, apparently scattered, setae and numerous spines, the last arranged in definite clusters dorsally and on the abdomen ventrally, the median and marginal dorsal groups closely approaching each other, so that there appears to be an almost continuous transverse band of spines on each segment; ventral rows of spines on abdomen narrower, with the spines less numerous and more scattered; ring not unusual for the genus.

This species has been described from two mounted adult females from Elfers, Fla., on Spanish moss (*Tillandsia usneoides*) (Bromeliaceae), collected July 12, 1917, and forwarded by C. E. Wilson (holotype and paratype), and from six mounted adult females, five mounted larvæ, and a number of unmounted specimens from Motes Station, Fla., on *Tillandsia usneoides*, collected by H. W. Fogg, October, 1921, and July, 1922, and kindly forwarded for study by G. E. Merrill of the Florida State Plant Board (paratypes). Material from Gainesville, Fla., collected July, 1924, by Hart and Merrill, has also been reported.

The types are in the United States National Collection of Coccidae. Paratypes are in the collection of the Florida State Plant Board.

ORTHEZIA ULTIMA COCKERELL

Figs. 4, B; 6, L; and 33; Pl. 2, G

REFERENCE. — Cockerell, 1902, Canad. Ent. 34: 88–89.

ADULT FEMALE.—Body of female, with secretion, about 1.5 millimeters

long by about 1.3 millimeters wide; ovisac usually ranging from 1.5 to about 3 millimeters long; body covered dorsally with white secretion, except for a narrow band on each side, this curved toward the median line at the posterior end, running most of the length of the body, but terminated anteriorly by the first lateral marginal plate and posteriorly by the terminal dorsal plates, the wax arranged in the usual marginal and dorsal plates but those of the dorsum not sharply differentiated, the anterior lateral plates short and stout, gradually increasing in length posteriorly and the posterior pair more or less distinctly longer than the remainder; body of female, as mounted, about 1.5 millimeters long by 1.3 millimeters wide, uniformly oval, or more or less distinctly tapering anteriorly; derm membranous, except for a definitely developed, very elongate oval, usually dis-

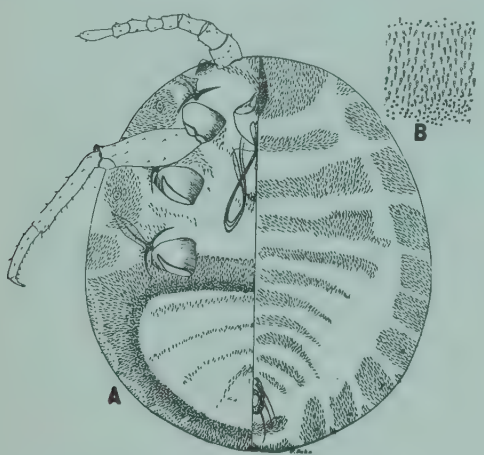


FIG. 33.—*Orthezia ultima*, adult female: A, body, dorsal and ventral, × about 31; B, section of anterior median portion of ovisac band; × about 60

tinctly chitinized, thickened plate running back along the dorsal median line from the anterior apex of the head, and an elongate thickening ventrally on each side of the genital opening; antennae normally 8-segmented, the segments short and stout as compared with practically all of the other species in the genus, measurements of those of one antenna in microns as follows: I, 79; II, 71; III, 94; IV, 66; V, 61; VI, 55; VII, 57; VIII, 125; spine, 21; eyestalk rather flat conical, apex rounded; legs short, in agreement with the antennal length, fairly stout, entirely characteristic for the genus, tarsal claw with 2 or, more rarely, 3 fairly distinct denticles on the inner face; beak rather stout conical, with an indistinct suggestion of a division near base; thoracic spiracles characteristic for the genus, opening in a spine cluster and with some spines close to the opening, but without an evidently developed spine collar; with 7 pairs of long tubular abdominal spiracles; derm pores of the usual quadrilocular disk type, occurring both dorsally and ventrally, most abundant in the inner portion of the ovisac band; derm with slender setae occurring occasionally both dorsally and ventrally; derm spines arranged in the usual 11 marginal and 10 dorsal clusters on each half of the body, the dorsal clusters distinctly forming transverse bands, but plainly and even rather widely separated from the corresponding marginal clusters, particularly on the abdomen, this clear area accounting for the narrow bare stripe visible between the dorsal and marginal plates of secretion in the perfect specimens; ovisac band broad, interrupted posteriorly, composed of numerous spines and crowded with disk pores along the inner margin and through the inner fourth; band inclosing 4 loose and narrow transverse rows of spines, all of these, except the last, extending well toward the ovisac band on each side; anal ring characteristic for the genus; bands of pores on each side joined, or nearly so, at the ends, the inner margin of each distinctly but not prominently angled anteriorly and posteriorly; ring with the usual 6 setae.

This species has been redescribed from the following material: From Ceres, Santa Fe Province, Argentina, probably on a composite, collected by L. Bruner in 1902 (type). It does not appear to have been observed since originally collected.

ORTHEZIA URTICAE (LINNAEUS)

Figs. 4, C; 6, M; 7, O; and 34; Pl. 2, H

REFERENCE.—Linnaeus, 1758, Systema Naturæ, ed. 10, p. 453 (as Aphis).

SYNONYMS.—*Orthezia characias* Bosc, *Dorthezia dispar* Kalt., *Coccus dubius* Fabr., *Aphis urticata* Stewart (see Fernald, 1903, Cat. Cocc. World, p. 36); *Dorthezia delavauxii* Thieb., "*Coccus glecomae* Fabr." Dougl. (see Lindinger, 1912, Die Schildläuse, p. 367, and Fernald, 1903, Cat. Cocc. World, p. 34); *Orthezia maenariensis* Dougl. (see Laing, 1922, Ent. Mo. Mag. 58: 254); *Orthezia japonica* Kuw., *Orthezia martelli* Leon.

ADULT FEMALE.—Total length of body with ovisac, in material examined, about 5 millimeters, but as much as 10 millimeters according to published descriptions; total length of body with caudal plates about 3.5 millimeters; ovisac variable; width of body with secretion 2.5 millimeters; body completely covered dorsally with well-developed plates of secretion, these

fairly distinctly segregated into the usual marginal and dorsal plates, the anterior marginal tufts relatively short and stout, increasing in length and becoming more slender and fingerlike posteriorly, anterior dorsal paired tufts, at most, only indistinctly divided along the median line, the remaining dorsal pairs distinctly separated by a triangular groove, dorsal tufts transverse, each somewhat pointed nearer the middle than the margin, and the outer third of each tuft very often separated from the remainder by a light diagonal impressed line, this producing the appearance of an additional separate tuft of secretion between the corresponding median and marginal tufts, this appearance, however, not correlated with any corresponding segregation of the spines in the dorsal spine bands of the body; ovisac strongly ribbed dorsally; body closely resembling *O. solidaginis* in morphological characters, differing noticeably only in the following details: Length of body as mounted 3.25 millimeters, width 2. 5 millimeters; measurements of segments of one antenna variable, in microns as follows: I, 172; II, 150; III, 185; IV, 160; V, 136; VI, 111; VII, 114; VIII, 196; eyestalk rather elongate conical, nearly always bearing a very distinct lateral tubercle on the anterior margin, rarely with 2 such tubercles; tarsal claw usually with 2, more rarely with 3, not very distinct denticles on the inner face; collar of spines surrounding opening of each thoracic spiracle even more developed and more conspicuous than in *solidaginis*, the chitinous rim supporting the spines completely encircling the opening of the spiracle; ovisac band resembling that of *solidaginis* closely, except that in the section between and immediately behind the posterior legs the disk pores that are distributed through the posterior third of the band in *solidaginis* occur along the posterior margin in *urticae*, although distributed through the lateral section of the ovisac band much as in *solidaginis*.

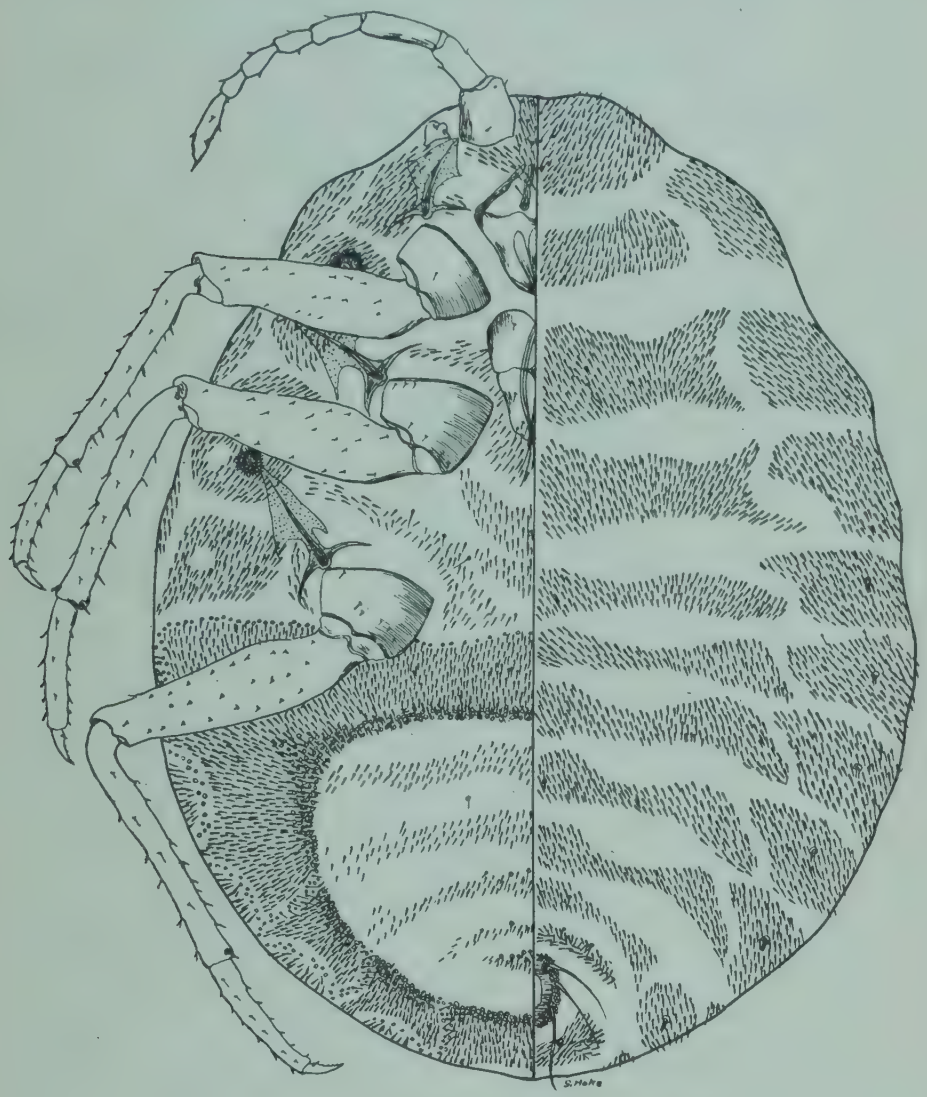


FIG. 34.—*Orthezia urticae:* Adult female, body, dorsal and ventral; × about 31

This species has been redescribed from material from France, Prussia, and Askhabad, Transcaspia, Central Asia. The published distribution records include Algeria, Austria, Bohemia, Corfu, England, France, Germany, Greece, Italy, Moravia, and Tyrol.

The synonyms listed for this species, excepting two only, have been accepted on the basis of published references, mostly in European literature. The writer has had an opportunity to examine specimens of an Orthezia collected by Prof. T. D. A. Cockerell at Tsuruga, Japan, on *Medicago denticulata* (Leguminosae) which appear to agree entirely with Kuwana's description of *Orthezia japonica*, and which it has not been possible to separate from *O. urticae*, all of the presumably important characters agreeing very closely with those of European specimens of that species available for comparison. Through the great kindness of Dr. F. Silvestri the writer has also been able to examine a single adult female from the type material of *Orthezia martelli* Leon., with the result that this species is, in his opinion, identical with *O. urticae*. It should be noted, however, that this conclusion has been based on the examination of a single decidedly imperfect specimen.

The known host records include Achillea (Compositae), Aegopodium (Umbelliferae), Artemisia (Compositae), Aster (Compositae), Ballota (Labiatae), Caltha (Ranunculaceae), Centaurea (Compositae), Compositae, Coronilla (Leguminosae), Cuscuta (Convolvulaceae), Erica (Ericaceae), Euphorbia (Euphorbiaceae), Galium (Rubiaceae), Geranium (Geraniaceae), Glaux (Primulaceae), Glecoma (Labiatae), Gramineae, Hedera (Araliaceae), Heracleum (Umbelliferae), Hieraceum (Compositae), Humulus (Moraceae), Labiatae, Lathyrus (Leguminosae), Leontodon (Compositae), Matricaria (Compositae), Melampyrum (Scrophulariaceae), Melittis (Labiatae), Onobrychis (Leguminosae), Parietaria, (Urticaceae) Phlomis (Labiatae), Rubus (Rosaceae), Stellaria (Caryophyllaceae), Symphytum (Boraginaceae), Taraxacum (Compositae); Teucrium (Labiatae), Trifolium (Leguminosae), Tunica (Caryophyllaceae), Urtica (Urticaceae), Vinca (Apocynaceae).

ORTHEZIA VARIPES (LEONARDI)

Figs. 4, D; 6, K; and 35

REFERENCE.—Leonardi, 1911, Bol. Lab. Zool. Gen. e Agr. R. Scu. Sup. Portici 5: 240–243, fig. 2, 3.

Through the courtesy of Dr. F. Silvestri the writer has been permitted to examine a single specimen from the type material of this species. The condition of the microscopic mount prepared from this specimen is such that no satisfactory outline drawing showing the position and relation of the body structures can be prepared. However, it can be determined that the species is extremely closely related to *O. ultima* Cockerell, differing positively, so far as the writer has observed, only in that the anterior median section of the ovisac band has disk pores distributed through the inner two-thirds of its width, in that there is not an evident single row of disk pores along the anterior margin of the band, and in that, in this section at least, the row of disk pores along the inner or posterior margin is not more than two deep while it is as much as three or four deep in *ultima*. It seems possible that the examination of additional and better material of both species may demonstrate that they are identical. As nearly as can be determined, the arrangement of the spine clusters and bands is practically identical with that found in *ultima*, and it is necessary to remark particularly on the fact that these, as shown in Leonardi's drawing of the denuded adult female, are very incomplete and inaccurate. The species was described from Cacheuta, Argentine Republic, collected on *Atriplex lampa* (Chenopodiaceae).

FIG. 35.—*Ortheziavaripes:* Adult female, section of anterior median portion of ovisac band; × about 115

ORTHEZIA YASHUSHII KUWANA

REFERENCE.—Kuwana, 1923, Dept. Agr. and Com. [Japan] Imp. Plant Quar. Sta. Bul. 3: 58–63, fig. 5, pl. 14.

An extended description of this species, accompanied by a number of figures, appeared in the above publication, the edition of which was almost totally destroyed by the Japanese earthquake of September, 1923. From the description it appears to the present writer that this species, on comparative study, may prove to have been based on nothing more than small-sized or somewhat stunted adults of *O. urticae*. It has not been possible to include it in the key. The species

was described from two of the larger islands of Japan and was recorded as occurring on wild chrysantheum (Compositae) and *Artemisia vulgaris* (Compositae).

SUBGENUS ARCTORTHEZIA COCKERELL

REFERENCE.—Cockerell, 1902, Entomologist 35: 114, 259. (As a section.)

The structural and other characters of this subgenus agree with those given in the generic diagnosis, except for the differences already emphasized in the key to the subgenera. The subgenus includes two well known species, one of which, as indicated by the map accompanying the discussion of it, has a holarctic distribution, while the other is thus far known only from the Western States and mostly, but not entirely, from rather high altitudes.

The host relationships of the two included species, so far as they are known, do not appear to possess particular significance.

The two species placed here may be separated by the following key:

KEY TO SPECIES OF THE SUBGENUS ARCTORTHEZIA

a. Median triangular spine groups relatively large, extending the full depth of the segment bearing each and completely isolating the two halves of the dorsal spine band on each; corresponding dorsal secretionary plates of perfect insect large, distinctly overlapping each other or the following segment ---------- *occidentalis* Douglas.

aa. Median triangular spine groups much smaller, extending over only about the anterior half of the depth of the segment bearing each and not isolating the halves of the dorsal band; corresponding secretionary plates of the perfect insect small, not reaching the following segment in any case ---------- *cataphracta* (Shaw).

ORTHEZIA CATAPHRACTA (SHAW)

Figs. 3, E; 6, N; 7, P; 36 and 37; Pl. 2, I

REFERENCE.—Shaw, 1794, Naturalist's Miscellany 5: 182.

SYNONYMS.—*Dorthezia chiton* Zetterstedt, *Orthezia signoreti* F. B. White, and *Orthezia uva* Blanchard (see Fernald, 1903, Cat. Cocc. World, p. 33).

ADULT FEMALE.—Broad oval, tapering somewhat anteriorly, size variable, length with secretion about 3.5 millimeters, width 2.5 to 3 millimeters; completely covered dorsally and ventrally with heavy plates of white secretion, except at the insertions of the appendages; ovisac short and broad, parallel-sided, extending about 1.5 to 2 millimeters beyond apex of body; dorsal wax plates complete, each half with 10 visible marginal plates, including the fused anterior median one, and 9 visible median transverse plates, all

FIG. 36.—*Orthezia cataphracta:* Adult female, body, dorsal and ventral; × about 31

paired except the posterior one, these occupying most of the dorsal area; differing noticeably from the other members of the genus, except *occidentalis*, in the possession of 3 unevenly diamond-shaped or triangular wedges of secretion along the middle line, one on each half of the last 2 thoracic and the first abdominal segments; body without secretion averaging 2 to 2.5 millimeters in length and 1.5 to 2 millimeters in width; stout oval, posterior end broadly rounded, anterior end somewhat tapering; derm membranous, except for appendages and a slightly chitinized thickening ventrally on each side of the genital opening; antennae normally 8-seg-

mented, all of the segments relatively short and stout; average lengths of the different segments in microns as follows: I, 196; II, 139; III, 89; IV, 51; V, 68; VI, 71; VII, 71, VIII, 214; eyestalk strongly produced, curved, thumblike, not distinctly conical; legs characteristic for the genus; tarsal digitules short, stout, spinelike, tarsal claw without denticle; beak stout conical, 1-segmented, the joint figured by List [6] in his elaborate monograph of the species not found in the specimens available for examination; thoracic spiracles not unusual, the openings placed rather close to the bases of the anterior and intermediate legs, not distinctly surrounded by a collar of spines; with 7 pairs of short tubular, abdominal spiracles; derm pores, so far as observed, of the quadrilocular disk type only, these scattered, rather rare on the dorsal surface, most noticeable in the interspaces between the bands of spines, more abundant ventrally in rather definite transverse bands, alternating with the spine bands, in the region inclosed by the ovisac band; none of these pores very heavily chitinized; body bearing inconspicuous, slender setae, scattered, or in indefinite rows, and very numerous, rather stout spines in bands corresponding to the superficial secretion and about as figured, this including 10 marginal clusters, counting the anterior one, and 10 dorsal clusters on each half of the body, the last, just anterior to the anal ring, quite small, and its secretion concealed from view by the posterior unpaired plate formed by the spine cluster just preceding it; the 3 small triangular plates of secretion on the middle line represented by 3 indistinctly isolated, small, triangular clusters of spines not wholly interrupting the transverse spine bands in which they occur; ovisac band composed of spines only, relatively narrow as compared with many of the species in the genus, the lateral portions cut by clear transverse lines; band inclosing 3 definite, continuous, transverse bands of spines, of which the anterior is much the heaviest and continued for its full width nearly to the ovisac band at each end, while the 2 posterior taper laterally and terminate before attaining the ovisac band; anal ring stout oval, more or less distinctly pointed posteriorly, with numerous pores, conspicuously angulate internally on each half, and with the usual 6 anal ring setae, these of moderate length.

This species has been redescribed from the following material: From Cooper Island of the Commander Islands, off Siberia, taken from the gullet of *Leucosticte griseonucha* a "rosy finch," by Dr. L. Stejneger, June 28, 1883, and from St. Anthony, Newfoundland, on *Iris setosa* (Iridaceae), collected at quarantine, Washington, D. C., by H. L. Sanford, September 15, 1916. The published European and other distribution records include "among the Alps," Austria, Bohemia, England, Greenland, Ireland, Lapland, Norway, Scotland, Siberia, Styria, Sweden, and Switzerland. The published host records include Calluna (Ericaceae), Carex (Cyperaceae), Geranium (Geraniaceae), grass (not specified) (Gramineae), Hymogyne (Compositae), mosses (not specified) (Musci), Rhacomitrium (Musci), Saxifraga (Saxafragaceae), Soldanella (Primulaceae), Sphagnum (Musci).

FIG. 37.—*Orthezia cataphracta:* Map showing actual known distribution. Large dots show records based on specimens actually examined; small dots show records based on published reports of occurrence

ORTHEZIA OCCIDENTALIS DOUGLAS

Figs. 4, F; 6, O; and 38; Pl. 2, K

REFERENCE.—Douglas, 1891, Ent. Mo. Mag. 27: 245.

SYNONYM.—*Orthezia californica* Ehrhorn (see Ferris, 1920, Stanf. Univ. Pub. Univ. Ser. Biol. Sci. 1: 13.

ADULT FEMALE.—Body, with secretion, fairly large, total length to end of

[6] List, J. H. Ztschr. Wiss. Zool. 45: 201–286, pl. 1–6, 1887.

caudal appendages 4.5 millimeters, total width 3.25 millimeters; body completely covered with dense, sharply defined wax plates, these occurring in the usual marginal and dorsal tufts, white or variously discolored, sometimes appearing yellow-brown or gray, but with only 10 marginal tufts, instead of the 11 found in typical Orthezia, these all more or less definitely flattened, the lateral and posterior ones curved and directed backward and from one another, the first 3 by large flattened, triangular, overlapping plates set on the median line, the remainder by a triangular depression, the last visible pair of dorsal tufts fused into a single plate; body, as mounted, stout oval, length about 3.5 millimeters, width about 3 millimeters, derm membranous; antennae normally 8-segmented, lengths of one in microns as follows: I, about 250; II, 215; III, 121; IV, 100; V, 89; VI, 93; VII, 111; VIII, 235; spine, 21;

FIG. 38.—*Orthezia occidentalis:* Adult female, body, dorsal and ventral; × about 31

downward, gradually increasing in length posteriorly so that the apical 3 or 4 overlap the ovisac to a marked degree; ovisac short and stout, not much, if any, longer than the body, distinctly ribbed dorsally; anterior pair of dorsal plates fused into a single transverse lobe, the members of each of the following 7 pairs distinctly separated eyestalk strongly tuberculate, fingerlike, curved, length through longitudinal axis about 162 microns; width about 72 microns; each placed immediately adjacent to a basal antennal segment; legs stout, the setae on the different parts heavy and spinelike, tarsal claw without any distinct denticles on the inner face, although fre-

quently very slightly thickened at a point corresponding with the usual location of the outer denticle; beak rather long conical, 1-segmented, with a faint suggestion of a joint sometimes visible near the base; thoracic spiracles not unusual, each opening in a spine cluster, without a definite spine collar around the opening, but with a fairly distinct band of disk pores surrounding each opening; with 7 pairs of very short tubular abdominal spiracles; derm pores of the usual quadrilocular disk type only, these occurring both dorsally and ventrally, particularly in the interspaces between the spine bands; derm with scattered, slender setae both dorsally and ventrally; derm spines arranged in 10 marginal and 10 dorsal, compact, closely crowded clusters, with the second, third, and fourth dorsal bands completely interrupted medially by broad, triangular, similarly crowded spine clusters set off from the remainder of the band on each side by narrow diagonal clear strips, these last clusters evidently forming the 3 triangular wax plates of the perfect insect; ventral ovisac band stout anteriorly, somewhat narrowed laterally and interrupted by several clear spaces; anal ring oval, with the pore bands on each half united at anterior and posterior apices, and each very strongly angulate along the inner margin at three points, one opposite each anal ring seta; the usual 6 anal ring setae rather long and slender.

This species has been redescribed from the following material: West Cliff, Custer County, Colo., in nests of *Formica integra*, coll. T. D. A. Cockerell, 1889 (cotype); Beulah, N. Mex., coll. T. D. A. Cockerell, March, 1900; the same, no date, coll. Miss W. Porter; Kaslo, British Columbia, on roots of trees and grass amongst rotten wood, coll. J. W. Cockle, April and May, 1908; Corte Madera Creek, Stanford University, Calif., on *Eriophyllum confertifolium* (Compositae), coll. M. N. Reeper, October, 1916; Boulder, Colo., on *Castilleja* sp. (Scrophulariaceae), coll. H. F. Dietz, June 8 and 12, 1917; near Eldorado, Colo. (9,000 feet), coll. T. D. A. Cockerell, October 1, 1922. The following are the additional published distribution records for the species: Colorado, near Fort Collins; New Mexico, at Trout Springs; California, in Marin County. There are no other published host records, although the species has been reported as occurring in ants' nests.

GENUS NEWSTEADIA GREEN

REFERENCE.—Green, 1902, Ent. Mo. Mag. 38: 284–285.

The following diagnosis may be given for this genus:

ADULT FEMALE.—Completely covered externally with depressed or flattened tufts or plates of secretion, much as in Orthezia; ovisac, when fully developed, about as long as the body; derm without definite chitinization except for a transverse median plate in the posterior ventral abdominal region in one species; antennae normally 6 to 7 segmented, the 2 basal segments very much enlarged and elongated as compared with Orthezia; eyestalk elongate, curved, fingerlike; legs similar to those of Orthezia, except for the complete fusion of the tibia and tarsus in each, the joint indicated only as a more or less distinct transverse line near base; beak 1-segmented, rather elongate conical, rounded at apex; with the usual 2 pairs of thoracic spiracles and with 5 anterior pairs of abdominal spiracles, the posterior pairs wanting; derm pores of the quadrilocular disk type; derm setae occurring occasionally over body and in a distinct cluster ventrally, anterior to the genital opening; body spines arranged in marginal and dorsal clusters as in Orthezia, but most of the marginal abdominal clusters fused into a conspicuous band; anal ring with the usual pore bands and 6 setae, the latter rather short, stout, and blunt-tipped as compared with Orthezia.

LARVA.—Very similar to that of Orthezia, differing primarily in the possession of only 4-segmented antennae.

ADULT MALE.—Not exhibiting any characters that will plainly segregate males of this genus from those of Orthezia.

This genus has previously been represented by only a single species, this occurring naturally in various parts of Europe, and, presumably through accidental introduction and under rather peculiar circumstances, in one place in Australia. A second, American, species is described below. This genus, excluding the Australian record, therefore has a holarctic distribution, and a majority of the records appear to be from northern localities or rather high altitudes, an apparently conspicuous exception to this being the principal record on which the new American species is based, this being from an altitude only a little above sea level, and latitude 39 degrees north. The original collector of the species has

suggested, however, that it was possibly introduced at this point from a higher altitude through deposition of driftwood or other trash from upstream, and its recent discovery in such a higher altitude, toward the headwaters of the stream along which it was first found, tends to strengthen this hypothesis.

The host relations of the species of the genus are not altogether certain, but it seems probable that its members may feed either on fungus hyphae of the types that form long rootlike cords extending over and through rotted wood, or on the more or less exposed roots of higher plants.

The two species now included in this genus may be separated by the following key:

KEY TO SPECIES OF THE GENUS NEWSTEADIA

a. Antennae normally 6-segmented; no elongate, transverse, chitinous thickening ventrally and posteriorly just inside ovisac band; intermediate dorsal abdominal spine bands narrow, constricted, but not definitely interrupted where crossing the median line; length about 1.6 mm ..... *floccosa* (De Geer).

aa. Antennae normally 7-segmented; with a distinct, elongate, transverse, chitinous thickening ventrally and posteriorly just inside ovisac band; intermediate dorsal abdominal spine bands broader, strongly constricted and nearly, if not quite, interrupted over the median line; length about 2.7 mm ..... *americana*, new species.

NEWSTEADIA AMERICANA, NEW SPECIES

Figs. 39 and 40; Pl. 2, J

Occurring in moist situations beneath rotten bark, on rotten logs, and in trash; possibly feeding on the roots of trees, shrubs, or plants that had forced their way into the situation mentioned; no definite host or hosts assignable.

ADULT FEMALE.—Completely covered dorsally by heavy, definitely arranged plates of secretion, and at maturity secreting an ovisac equaling or sometimes exceeding the body in length; maximum length of body and ovisac secretion 6 millimeters, width 3 millimeters; dorsal secretion arranged as follows: 1 large, median, triangular plate projecting from the anterior apex of the body; 1 similar but much smaller just behind this; 7 large, transverse, paired median plates, separated by a fairly distinct median impressed division line, of which the second, third, and fourth from the anterior end are the largest and of which the first 3 protrude diagonally forward and upward, the fourth directly upward, and the remaining 3 diagonally outward and slightly backward, all these not standing up prominently, but rather flattened; posterior to the last paired plate with a relatively long and narrow single median wax plate protruding backward above the anal opening, and beneath this plate with a much smaller paired plate, with its parts often fused more or less distinctly to the single plate above and wholly concealed as the insect is viewed from above; lateral secretion, as distinct from that forming the ovisac, composed of 4 stout, curved, somewhat fingerlike plates, of which the posterior is much the longest and flattened and curved around the anterior ends of the ovisac; ovisac usually distinctly ribbed, the dorsal half made up of 8 overlapping ribs, ventral half made up of almost smooth or at most very finely striated secretion strongly curved to inclose numerous oval eggs, these pale yellow, at least after death; body ventrally likewise completely covered with secretion arranged in definite plates; body of female, as mounted on slide, short oval, sometimes nearly circular; average length 2.5 millimeters, average width 2.2 millimeters; derm membranous except for appendages and a single, elongate, transverse, more or less distinctly chitinized ventral plate posterior to the genital opening; antennae normally 7-segmented, rarely 5- or 6-segmented, the basal segment very long and stout, the second segment about as long as the first, but much more slender, and more or less distinctly constricted just before the apex, the remaining segments much shorter and progressively shorter up to the next to the last, apical segment about as long as second and with a long slender spine and 1 stout seta at its tip, the average length of the different segments in microns as follows: I, 268; II, 221; III, 61; IV, 54; V, 46; VI, 46; VII, 196; apical spine, 114; various antennal segments with a number of slender spines, those of the intermediate segments on the apical margins of each and those on the remaining segments more or less scattered through the length except on the basal, here more

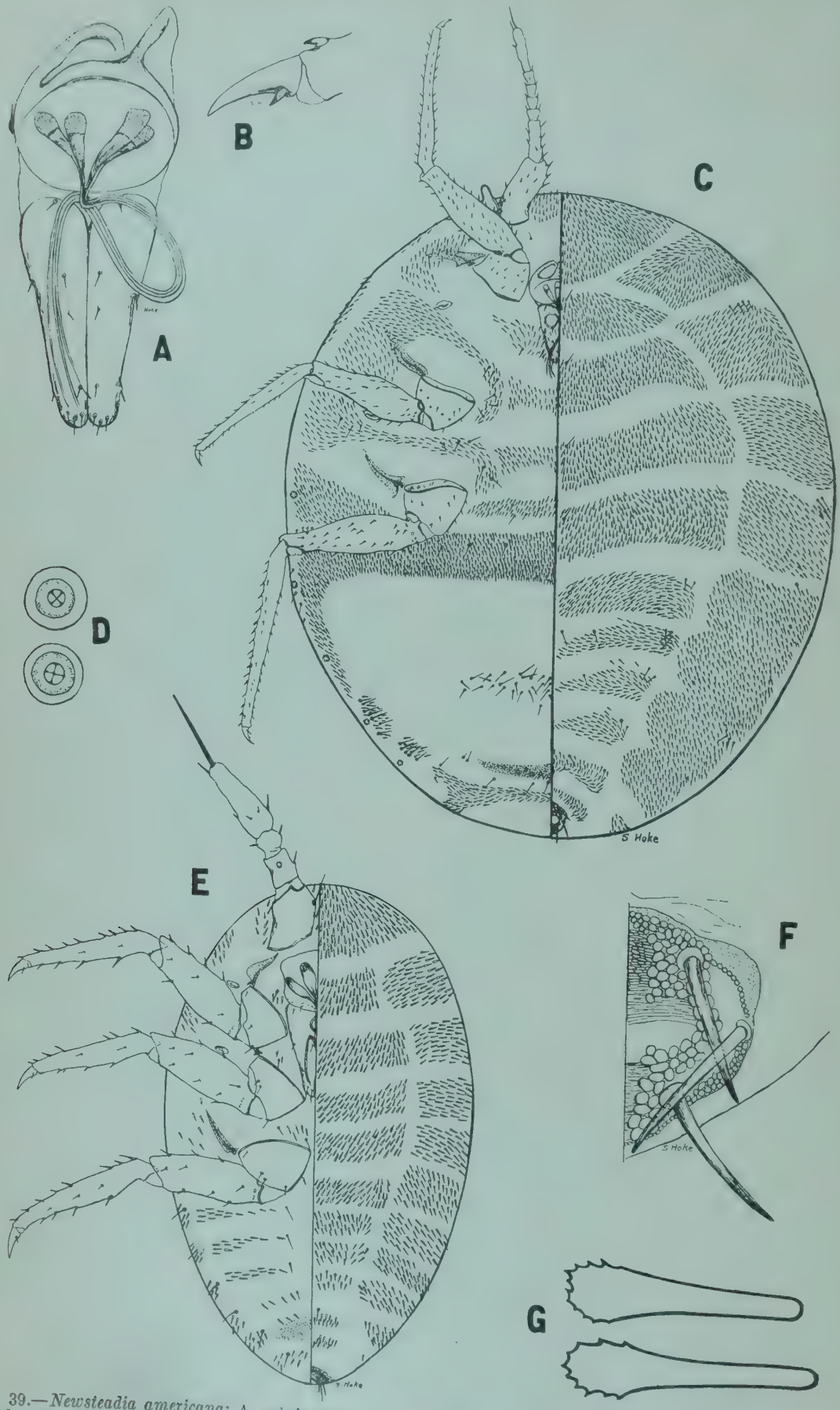


FIG. 39.—*Newsteadia americana:* A, adult female, beak, × 120; B, same, tarsal claw, × 230; C, same, body, dorsal and ventral, × about 31; D, same, quadrilocular disk pores, × 1,500; E, larva, body, dorsal and ventral, × 230; F, adult female, anal ring, showing pores and setæ, × 335; G, same, dorsal spines; × 1,500.

slender and occurring on the apical half only; eyestalk elongated, almost fingerlike, slightly curved; average length 114 microns, width at base 68 microns; legs fairly large and stout, the joints between trochanter and femur and between tibia and tarsus completely obliterated except only for a diagonal line near the base of the latter combination, parts bearing numerous, but not grouped or crowded, slender spines, these appearing to have a more or less definite arrangement on the combined tibia and tarsus; tarsal claw slender, only slightly curved and enlarged at base, without denticles; tarsal digitules short spines, not more than one-eighth the length of the claw; trochanter with 3 to 4 oval pores on each face; beak 1-segmented, elongate, only slightly tapering; somewhat expanded close to base, bearing a few slender spines at its tip, average length 232 microns, width 168 microns; thoracic spiracles

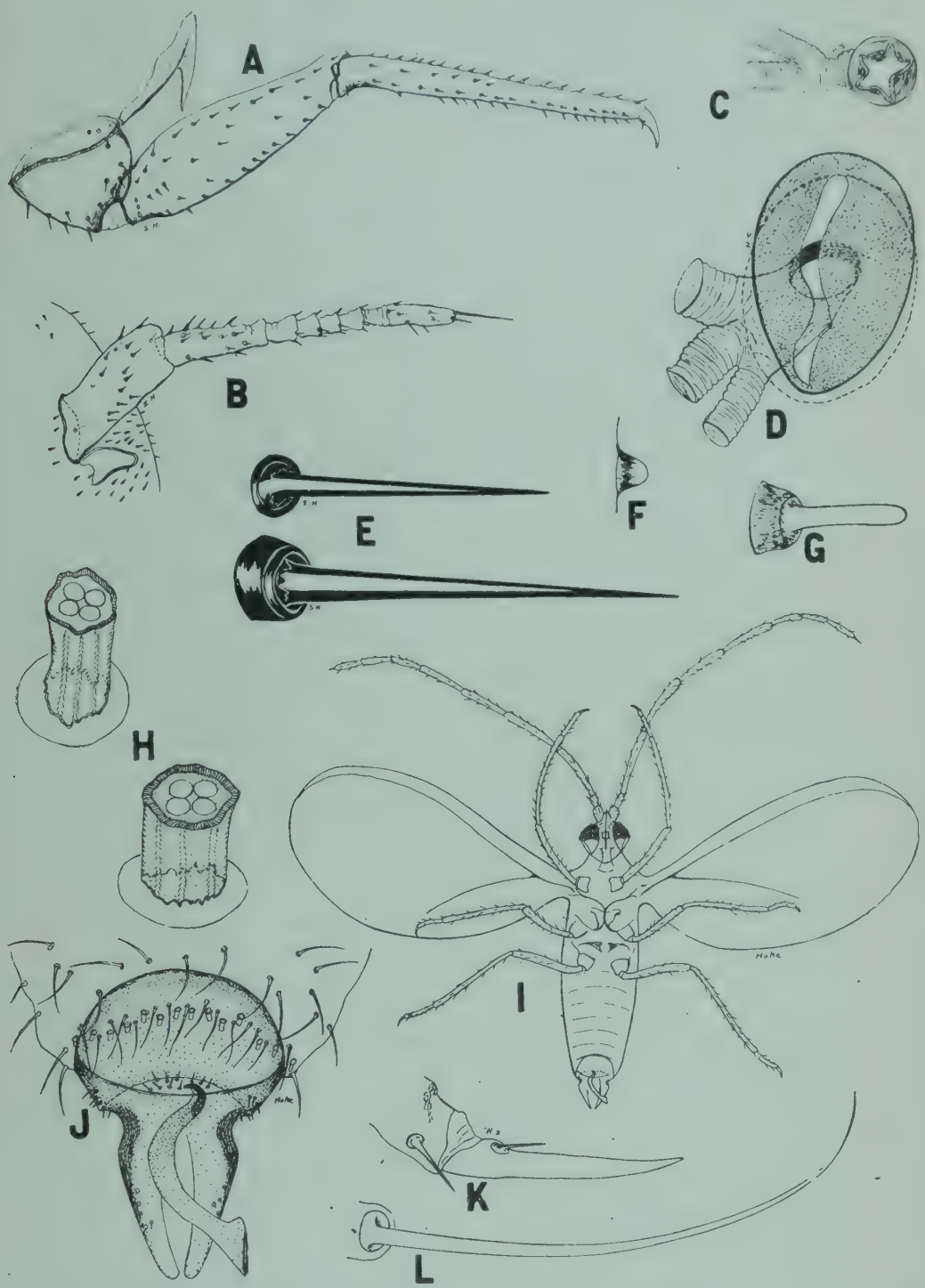


FIG. 40.—*Newsteadia americana:* A, adult female, leg, × 60; B, same, antennae, × 60; C, same, abdominal spiracle, × 650; D, same, thoracic spiracle, × 650; E, same, body setae, × 1,500; F, adult male, tubercle on apex of abdomen, × 1,500; G, same, spine from apical appendage, × 1,500; H, same, tubular pores from row near apex of abdomen, × 1,500; I, same, outline, × 20; J, same, apex of abdomen, × 230; K, same, tarsal claw, × 540; L, same, abdominal seta; × 1,500

not unusual, external opening a wavy, elongate-slit, without spines, but with a few quadrilocular disk pores scattered around this opening; abdominal spiracles much smaller than the thoracic, external opening usually irregularly star-shaped, each accompanied by a number of quadrilocular disk pores, these spiracles occurring, so far as can be determined after a careful examination of available material, in 5 pairs, the 3 posterior pairs usually found in Orthezia apparently lacking in this species; derm with the quadrilocular disk type of pore only, these scattered over the body, but most abundant ventrally in the genital region, and dorsally in the spaces between the spine clusters, with smaller quadrilocular pores, set at the inner ends of tubular ducts, scattered through both the dorsal and ventral spine clusters; spiracles with a more or less distinct collar of irregular wax pores; derm ventrally with a transverse cluster of stout setae anterior to the genital opening, and a few additional elsewhere in connection with spine clusters; dorsally with a few such setae, usually in more or less definite relation to the clusters of spines; body spines in clusters dorsally and laterally, corresponding approximately in size and shape to the external secretion already described, ventrally in definite clusters surrounding the legs, antennae, and beak, and in a definite transverse band across the abdomen just behind the posterior legs (ovisac band), this continuing posteriorly at the margin for a short distance, finally fading out, not continued as a broad band completely encircling the ventral surface of the abdomen, as is usual in Orthezia; inclosed ventral surface of the abdomen without spines; anal ring of moderate size, with an outer row and an inner row of irregularly clustered pores, and 6 relatively short and stout setae.

LARVA.—Elongate oval, tapering somewhat posteriorly, average length as mounted 140 microns, width 86 microns; derm membranous except for appendages, and a transverse plate ventrally just anterior to the anal ring; antennae 4-segmented, the first stoutest, the last longest, and with an elongate terminal spine as in adult, each segment bearing a few setae and the terminal, in addition, bearing a curved fingerlike spine near base; legs not unusual, the fusion of segments as in adult; derm bearing numerous spines, these grouped into definite clusters approximately as indicated in figure; midventral longitudinal area entirely destitute of spines or of setae and the lateral ventral area with only narrow bands of setae; spines dorsally grouped into median transverse clusters on the head and thorax and into similar but shorter and bisected rows on the abdomen; abdominal spiracles present, minute, number not definitely determined; with a relatively large quadrilocular disk pore close to the opening of each; derm with an occasional quadrilocular disk pore, and with an occasional relatively large seta, the latter in definite relation to the spine groups; anal ring in general similar to that of adult.

ADULT MALE.—Small, elongate; total length 1.4 millimeters, width through thoracic region about 0.5 millimeter, length of antenna about 2.1 millimeters, length of wing 1.7 millimeters, width 0.9 millimeter; head strongly triangular in outline anteriorly, bearing a number of elongate slender hairs; compound eyes large, slightly protruding; with 2 ocelli, 1 behind and somewhat above the posterior outer angle of each compound eye; head broad behind the eyes, and nearly as wide as the distance through the eyes; antennae 9-segmented, the first 2 stout, the remainder very elongate linear and, excepting the 2 basal, bearing an apparently varying number of slender, threadlike setae, and in addition numbers of long, slender setae, these never in clusters or in any obviously definite arrangement, terminal segment with a stout, curved seta close to apex; legs elongate, slender, the trochanter distinctly set off from the femur, and the tibia and tarsus not fused as in other stages of the species, claw very long and slender, only slightly curved, digitules as in adult but more slender; legs bearing numbers of long, very slender setae on the basal half, these becoming stout on the apical one-half to one-third of the tibia and on the tarsus; wing broad, with only the costal thickening arising close to the base; halteres not observed; abdomen roughly parallel-sided, bearing transverse segmental rows of long slender setae, and an indeterminate number, apparently 5, of simple abdominal spiracles; with a single transverse band of stout, tubular ducts just anterior to the apex, and with very peculiar chitinized paired terminal appendages instead of the fused conical sheath present in most male coccids.

This species has been described from several mounted and unmounted adult females, larvae, and males from the Virginia shore of the Potomac River opposite Plummers Island, Maryland, under rotting logs and among trash,

collected by H. S. Barber, August 8, 1917 (holotype and paratypes); from the same locality, collected by H. S. Barber and Harold Morrison, June 15, 1922 (paratypes); and from Grimsby, Ontario, Canada, collected by Horn (paratype).

The types are in the United States National Collection of Coccidae.

NEWSTEADIA FLOCCOSA (DE GEER)

REFERENCE.—De Geer, 1778, Mem. Hist. Inst. 7: 604–605.

This species has been included in the key and the generic discussion on the basis of an examination of specimens of the adult female from England, Newstead coll., and from Loch Katrina, Scotland, among moss (Musci), coll. T. D. A. Cockerell, June 17, 1921. Published records include, in addition to the general localities given above, Australia (presumably accidentally introduced), Bohemia, France, Germany, and Sweden. The reported hosts are Cyperaceae, Glecoma (Labiatae), Gramineae, Helianthemum (Cistaceae), Luzula (Juncaceae), mosses (Musci), Sphagnum (Musci).

NEWSTEADIA TRISTANI (SILVESTRI)

REFERENCE.—Silvestri, 1924, Bol. R. Soc. Española Hist. Nat. 24: 174–176, figs. vii–ix (as Ortheziola).

Thanks to the numerous figures and extended description given by Doctor Silverstri, it is possible to reassign this form as the immature stage of some species of the genus Newsteadia. The external appearance, the number of abdominal spiracles, and the details of the body structure harmonize closely with the generic characters with the single exception of the number of antennal segments, and here it seems possible that the method of treatment for microscopic study may have influenced the appearance of the antennae, and that they actually have a greater number of segments than the three shown. The ventral ovisac band of the adult female is so striking a characteristic in all of the species in the subfamily that are positively known in this stage that it is hardly conceivable that the form described by Doctor Silvestri can be anything other than the preadult stage of some member of the genus. Certainly it can not be associated properly with either Ortheziola or Nipponorthezia.

No examples of this species have been available for study. It was described from San Jose, Costa Rica, where it was found in the ground in disintegrated vegetation.

GENUS MIXORTHEZIA, NEW GENUS

Adult female normally with plates of secretion in general resembling those of other genera, and with ovisac at maturity; body oval; antennae 4-segmented, the 2 terminal forming an elongate club; eyestalk small, irregularly conical, not fused to basal antennal segment; legs with tibia and tarsus fused in addition to coxa and trochanter, claw without denticles; beak elongate, 1-segmented; thoracic spiracles not unusual, abdominal occurring in 8 pairs as in Orthezia; derm pores quadrilocular, in 2 sizes, the larger often grouped in clusters of 2 to 5, the smaller at ends of tubular ducts; derm spines in bands and clusters as in other genera, but with the apices of each slightly enlarged and truncate; body setae relatively large and numerous; anal ring normal, with pores and 6 subequal setae; with a narrow, irregular, transverse thickening anterior to the anal ring dorsally.

Type of genus: Mixorthezia cubana, new species.

MIXORTHEZIA CUBANA, NEW SPECIES

Fig. 41

ADULT FEMALE.—All specimens alcoholic and the secretionary covering in consequence largely destroyed, but evidently consisting of flattened white wax plates corresponding in number and position to the clusters of spines and in general closely resembling those of other genera; ovisac probably shorter than the body; as mounted, oval, length 1.4 millimeters, width 0.9 millimeter; derm without chitinization except for a narrow transverse thickening, nearly obsolete across the middle line, just anterior to the anal ring; antennae normally 4-segmented, the first large, the second small, the last two forming a rather evident elongate club, apical spine long and relatively slender; eyestalk small, irregularly conical; legs not unusual, trochanter and femur completely fused, tibia and tarsus fused, but apparently with a division line indicated near the base of the combined part, claw long and slender, without denticles, tarsal digitules not differentiated from the spinelike setae present in rows on the part, claw digitules small, spinelike; beak well developed, length about 165 microns, width about 97 microns, 1-segmented; with the usual 2 pairs of thoracic spiracles, without spine collar around opening; with 8 pairs of short tubular, abdominal spiracles, the posterior pair on each side grouped as in

Orthezia; derm pores of the usual quadrilocular type, occurring singly, or frequently in clusters or strings of from 2 to 5, these pores relatively large and most numerous between the ends of the dorsal, marginal, and ventral spine bands; outer margin of ovisac band and anterior margins of transverse spine bands inclosed by ovisac band with a single row of much smaller quadrilocular disk pores set at inner ends of short tubular ducts; ovisac band with a scattered row of larger disk pores along outer margin of at least the anterior section; derm spines arranged in bands and clusters about as shown in figure, with 9 dorsal and 11 marginal groups on each side, the individual spines with very slightly swollen, more or less distinctly truncate apices; with a crowded cluster of small conical spines in the genital region; with the body setae relatively numerous and large, particularly in the ventral abdominal region; anal ring stout, nearly circular, with 6 setae and pores, but the exact character and arrangement of these not determinable from the material available.

PREADULT FEMALE.—Rather closely resembling the adult, except for the absence of the ovisac band and the reduction in the development of some of the structures associated with it.

No other stages have been available for examination.

The species has been described from two adult females, one mounted, the other unmounted, from four mounted preadult females, all collected at San Antonio do Sul, Oriente Province, Cuba, by Dr. W. M. Mann in 1920, and from another mounted immature female from Guantanamo, Oriente Province, Cuba, collected by Doctor Mann at approximately the same time.

The types are in the United States National Collection of Coccidae.

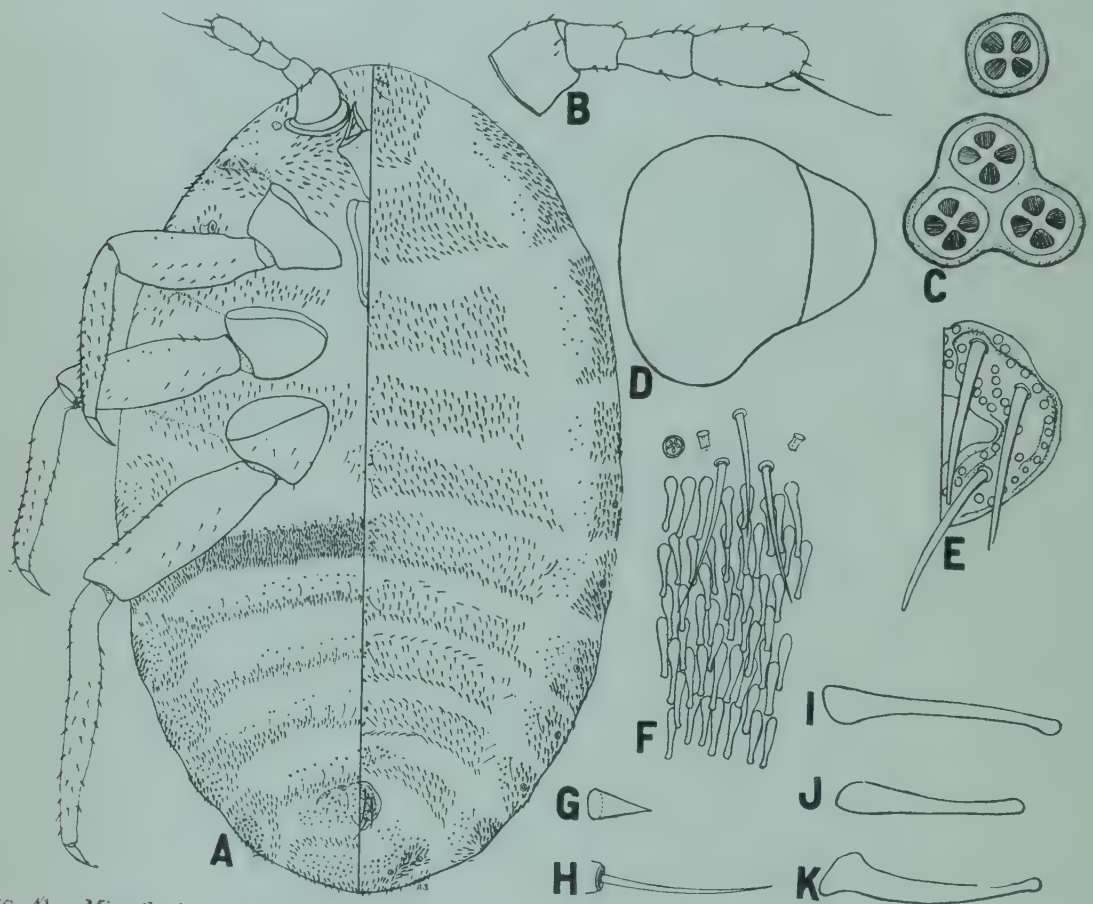


FIG. 41.—*Mixorthezia cubana*, adult female: A, body, dorsal and ventral, × 55; B, antenna (from preadult), × 115; C, quadrilocular disk pores, × 1,500; D, eyestalk, × 1,500; E, anal ring showing pores and setae (from preadult), × 500; F, section through anterior median portion of ovisac band, × 500; G, conical spine near posterior apex of body, × 1,500; H, body seta, × 500; I, dorsal spine, × 1,500; J, spine from ovisac band, × 1,500; K, spine from midventral abdominal area, × 1,500

GENUS ORTHEZIOLA SULC

REFERENCE.—Sulc, 1894, Sitzber. K. Bohm. Ges. Wiss. 44: 1.

This genus may be characterized as follows as regards the adult female: External appearance of body in general resembling that of other genera of the subfamily; that is, bearing plates

of wax secretion and a definite posterior ovisac; body stout oval; antennae actually 3- but apparently 4-segmented, the terminal much the longest, the apical spine quite long and slender; apex of eyestalk cylindrical, its base apparently enlarged and forming a pseudobasal antennal segment; legs with tibio-tarsal articulation wanting, the parts fused, the leg setae spinelike, in distinct rows on tibiotarsus; beak 1-segmented, with a suggestion of a joint near base; each of the two pairs of thoracic spiracles opening in a spine cluster; abdominal spiracles not located in single specimen available for examination; derm pores mostly of the usual quadrilocular disk type, some in ventral region larger, apparently with 8 loculi; derm spines stout, swollen at bases and mostly distinctly capitate at apices, some, in a small cluster on each side of anal ring, but ventrally, oval in outline; body setae relatively large, but slender; anal ring with pores and 6 subequal setae; with a conspicuous transverse platelike thickening dorsally anterior to the anal ring.

While the appearance of the antennae gives a very distinct impression that each is 4-segmented, the actual fact seems to be that each eyestalk is enlarged laterally in the form of a short chitinized cylinder, or is fused to such a dermal cylinder, which supports the antenna. This conclusion is apparently verified by the fact that the small, double-walled circular to oval pore, probably sensory in function, which, in the writer's experience, occurs constantly on or close to the distal margin of the second antennal segment is, in this species, found on what appears to be the third antennal segment. Shortly after the first publication of the description of this genus Maskell advanced the view that there were actually 4 segments present rather than 3, as given in the description. Not long after this Sulc published a reply (in Bohemian), advancing additional reasons, apparently perfectly sound, for considering the proper number of segments to be 3, and this last discussion has been made available to the present writer through the kindness of Mr. James Zetek, of the Ancon, Canal Zone, laboratory of the Bureau of Entomology, who translated all of its essential parts into English.

ORTHEZIOLA FODIENS GIARD

REFERENCE.—Giard, 1897, Comp. Rend. Soc. Biol. [Paris] 10: 583–585.

This species was described from the island of Guadeloupe from the roots of a coffee tree. No specimens have been available for examination, and the characters given in the brief description are inadequate as a basis for comparing this species with the others discussed here.

ORTHEZIOLA VEJDOVSKYI SULC

REFERENCE.—Sulc, 1895, Sitzber. K. Bohm. Ges. Wiss. for 1894, No. 44, p. 2.

SYNONYM.—*O. signoreti* Haller (see Lindinger, 1912, Die Schildläuse, p. 373).

This species has been included in this paper on the basis of an examination of a single adult female, collected on rose bush from Malines, Belgium, at quarantine, Washington, D. C., by R. D. Kennedy, May 16, 1922 (F. H. B. No. 33632). It has also been recorded from Bohemia, England, Switzerland, and the Madeira Islands, either in ants' nests, from moss (Musci), or from grass roots (Gramineae), and it seems almost certain that the true host in the record given above was not the rose bush as recorded, but rather the moss used as packing around the rose plants.

GENUS NIPPONORTHESIA KUWANA

REFERENCE.—Kuwana, 1916, Annotationes Zoologicae Japonensis 9: 150.

SYNONYM.—*Orthezinella* Silvestri, 1924, Bol. R. Soc. Española Hist. Nat. 24: 170.

This genus may be given the following diagnosis for the adult female: Superficial appearance much as in other Ortheziinae, actually resembling *Orthezia insignis*, body stout oval, derm membranous, antennae plainly 3-segmented; legs with the tibio-tarsal joint only very faintly suggested, these parts fused solidly; beak distinctly 2-segmented, long, only slightly tapering; with the usual 2 pairs of thoracic spiracles and with 6 pairs of abdominal spiracles, the two posterior pairs wanting; derm pores mostly of the usual quadrilocular disk type, but some in the ventral abdominal area larger and with 6 loculi; body spines enlarged at bases, with apices almost truncate, but not swollen; body setae relatively large but slender; anal ring with pores and 6 short setae, with the intermediate pair conspicuously smaller than the others.

NIPPONORTHEZIA ARDISIAE KUWANA

REFERENCE.—Kuwana, 1916, Annotationes Zoologicae Japonensis 9: 150–152, pl. 4, figs. 13–23.

In addition to cotype specimens kindly forwarded to the Bureau of Entomology by the describer of the species and collected at Yokohama, Japan, on *Ardisia japonica* (Myrsinaceae), the writer has examined a single specimen from Brooksville, Fla., on root of *Lawsonia inermis* (Lythraceae) collected by L. V. Bottimer at quarantine, Washington, D. C., Mar. 27, 1924 (F. H. B. No. 49655) and several specimens from Rockville, Pa., in ants' nest, collected by F. M. Trimble, February 14, 1921 (No. 0–105).

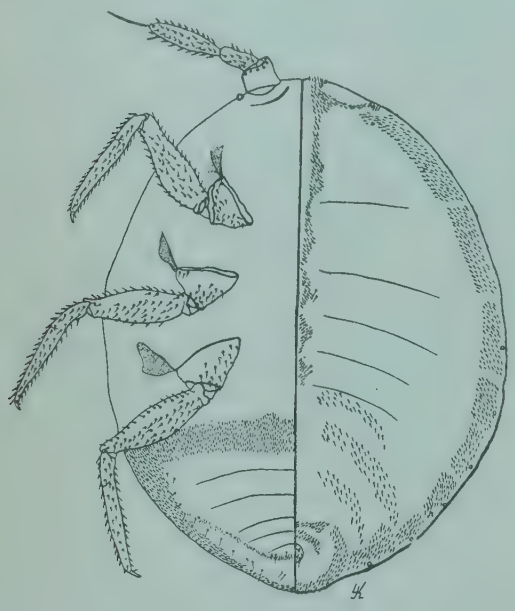


FIG. 42.—*Nipponorthezia ardisiae:* Adult female, body, dorsal and ventral; × about 25

The discovery of this curious coccid in the United States raises a question as to its probable native home, and as to whether it is as widely distributed naturally as *Orthezia cataphracta.* In a measure this discovery parallels the finding of another supposedly Japanese species, *Matsucoccus matsumurae* (Kuwana), apparently widely distributed through the Atlantic Coast region of this country. Until more is known regarding both of these species, this widespread and isolated distribution can hardly be looked upon as natural, and it suggests the possibility that the spread has been brought about through the movement of ornamental plants or nursery stock.

NIPPONORTHEZIA HISPANICA (SILVESTRI)

REFERENCE.—Silvestri, 1924, Bol. R. Soc. Española Hist. Nat. 24: 170–172, figs. i–iii (as Orthezinella).

If an exception be made of the single character of the number of antennal segments, then this species, as described by Doctor Silvestri, appears to agree closely with the genotype of Nipponorthezia in all generically important structural characters. Since neither the ovisac nor the ovisac band of spines is described or figured by Doctor Silvestri, it is evident that his specimens are preadult. It seems to the writer a possibility that the apparent division of the terminal portion of the antenna into 2 parts may be apparent only and due to some factor in the preservation and treatment of the specimens before study rather than to the actual presence of a joint. The species was described from Algeciras, Spain. No specimens have been available for examination.

NIPPONORTHEZIA NEOTROPICALIS (SILVESTRI)

REFERENCE.—Silvestri, 1924, Bol. R. Soc. Española Hist. Nat. 24: 172–174, figs. iv–vi (as Orthezinella).

The placing of this species in the genus Nipponorthezia is even less certain than was that of the preceding species, and it has been so placed largely because its describer considered it to be congeneric with *hispanica.*

The species was described from San José, Costa Rica. No specimens have been available for examination.

A TEST OF RAW ONIONS IN THE DIET AS A CONTROL MEASURE FOR WORMS IN DOGS[1]

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INTRODUCTION

Recently, Blanchasal (*3*)[2] has recommended that as soon as pups are old enough to take any food other than mother's milk they be fed two to three raw onions in minced meat or in hard-boiled egg with bread and milk added to make a fine paste. According to him, onions are anthelmintic when used raw, but not when cooked. This idea that onions are anthelmintic is not confined to veterinary medicine. Wolff (*13*) has recently noted that at Paramaribo, in Dutch Guiana, the British-Indian population has a low incidence of ascarid infestation (35 per cent) as compared with that of the negro population (76 per cent) and that of the creole population (58 per cent), and the suggestion is made by him that this lessened incidence may be due to an anthelmintic action of the onions, garlic, and other herbs which enter into the diet of the British Indians. The antiquity of this belief may be judged from the statement of Khalil (*6*) that Avicenna (this name being the Latinized form of Ibn Sina), who was born in 981 and died in 1037, recommended garlic, among other things, for "long worms." As further evidence it may be noted from an article in the Hospital and Health Review (*2*), on "Folk Lore in Medicine," that in Brittany the peasants wear as a protection against worms an amulet composed of cloves of garlic threaded together lengthwise on an ordinary string. This is worn as a collar and its charm is believed to act both as a preventive and as a cure for worms. Onion clysters are commonly recommended for pinworms.

The idea that articles of diet may have anthelmintic value is widespread and has received more or less attention from medical men. In many cases there is practically no evidence given to support the belief, but in other instances there is some available evidence in regard to the value of certain substances. Diet has been reported as a factor in the control of pinworms. Recently Nyberg (*8*) has recommended the use of fresh blueberries for oxyuriasis. Adults and large children are advised to eat half a liter of the fresh berries three or four times during the first day and once a day as a meal for the next six days. His original article is not available and his evidence in support of the idea that the treatment is of value can not be cited. Lutz (*7*) advised in oxyuriasis the use of a diet which would give the smallest possible residue. Along the same lines, Stettiner (*11*) reported that he had treated one patient for pinworm infestation for years with never more than transient benefit until the patient developed diabetes and carbohydrates were excluded from the diet. Following this the pinworms disappeared. Subsequently, he used an antidiabetic diet as an adjuvant to the usual measures for pinworm infestation and had prompt cures in four cases under this treatment.

The use of sugars has been recommended for the control of certain worms. A writer in the Pastoral Review (Melbourne, Australia) (*1*), reports the passage of large numbers of worms by horses after being fed several handfuls of coarse brown sugar. Richards (*9*) reports that at the suggestion of an Indian hospital attendant he fed large amounts of raw sugar to patients infested with the Guinea worm, *Dracunculus medinensis*, and found that if this was continued for two days the worm usually came out on the third day, being wound out easily and without rupturing. The treatment was successful in all cases, the worms coming away in the course of a few days instead of several weeks. In connection with filarids it may be mentioned that Robertson (*10*) believes that pepper and piperine have prophylactic value in food and therapeutic value in the treatment of established cases.

[1] Received for publication May 19, 1924; issued April, 1925.
[2] Reference is made by number (italic) to "literature cited," p. 159.

That parasitism and metabolism are subjects with many factors in common has recently been emphasized by Hall (*5*), and it is evident that the transition from ordinary food to drugs is a gradual one, passing through such inorganic substances as water and sodium chloride to the various inorganic drugs, and through the various vegetable foods containing small amounts of essential oils, alkaloids or glucosids to these substances as derived from plants containing relatively large amounts. In the transition one encounters large numbers of substances having at least feeble anthelmintic power and some with considerable anthelmintic potency. Thus tobacco may be added to the feed of sheep or poultry and if suitably used may have some little anthelmintic value, and pumpkin seed has considerable efficacy in removing tapeworms. The possibility, therefore, that raw onions fed for a considerable period of time might prove to have some anthelmintic value is not altogether remote on theoretical grounds. Unfortunately, the recommendations of Blanchasal (*3*) left the case still based on theoretical grounds, so far as any definite scientific evidence of the value of onions is concerned, and did not advance the subject distinctly beyond the stage of the onion amulet.

EXPERIMENTAL DATA

To obtain definite evidence on this subject to replace or at least to supplement the speculations and theory of physicians and veterinarians, the writers conducted an experiment as follows: Four dogs were fed daily on what may be called an onion Hamburger steak made up to contain 2 oz. of raw onion, which was regarded as the equivalent of one onion of average size, ground up with 1 lb. of raw or cooked meat for each dog daily. The first feeding was on February 27, 1924, and the experiment was continued for 60 days. Early in April it was noted that the dogs were not cleaning up this amount of food, and beginning April 9, after 43 days on the previous ration, the amount of meat was cut in half, the amount of onion being left as before. One dog, No. 658, was found dead on the thirty-seventh day of the experiment; the other dogs survived through the 60-day period and were then killed. The feces of all animals were examined daily for worms passed, but the feces for Saturdays and Sundays were not always kept separate. In the following protocols the worms found in the feces are reported for consecutive days, failure to find worms is expressed by 0, failure to pass feces is expressed by a hyphen (-), and the combined record for a Saturday and Sunday is given in parentheses, to call attention to the fact that this represents a two-day period. The protocols, showing worms passed but ignoring tapeworm segments, are as follows:

Dog No. 654: 0; 1 whipworm; 0; 0; 1 whipworm; 1 whipworm; 0; 0; 0; 1 hookworm; (0); 0; 0; 0; 0; 0; -; 0; 0; 1 whipworm; -; -; 5 whipworms; (0); 0; 0; 0; 0; 0; (0); 3 whipworms; 1 whipworm; 0; 3 whipworms; 2 whipworms; 0; 1 whipworm; 3 whipworms; 0; 0; 0; 0; 0; 1 whipworm; 0: 0; 0; (1 whipworm); 0; 0; 0; 0; 0; (1 whipworm);0. Total worms passed: 25 whipworms and 1 hookworm.

Dog No. 656: 1 whipworm; -; 0; -; 0; 7 whipworms; 1 hookworm; 1 whipworm; 0; 0; (0); 0; 0; 0; 0; 1 hookworm; 0; 0; 0; 0; -; 0; 0; (-); 0; 0; 0; 0; 0; (0); 0; 0; -; 0; 0; 0; 0; 0; 0; 0; 0; -; 0; 0; 0; -; (0); -; 0; 0; 0; 0; (0); 0. Total worms passed: 9 whipworms and 2 hookworms.

Dog No. 658: 0; 0; 0; 0; -; 0; -; 0; 1 ascarid; -; (0); -; 0; 0; -; 0; -; 0; 0; -; 0; 0; -; (0); -; 0; -; 0; -; (1 ascarid);-; -; -; 0. Total worms passed: 2 ascarids.

Dog 659: 1 whipworm; 0; 0; -; 1 whipworm, 1 ascarid; 4 whipworms; 0; -; 14 whipworms; (5 whipworms); -; 2 whipworms; 8 whipworms; 2 whipworms; -; 6 whipworms; 1 whipworm; 1 whipworm; 0; 1 whipworm; 6 whipworms; -; (1 whipworm); 0; 0; 0; 0; 0; (2 whipworms); 2 whipworms; 0; 0; 1 whipworm, 1 hookworm; 4 whipworms; 7 whipworms; -; 3 whipworms; 0; 1 whipworm; 1 whipworm; 9 whipworms; -; 0; 1 hookworm; 3 whipworms; 1 hookworm; (9 whipworms, 1 hookworm); -; 0; 1 whipworm, 1 hookworm; -; 0; 0; -; Total worms passed: 96 whipworms, 5 hookworms, and 1 ascarid. To this should be added 5 hookworms found in the large intestine postmortem, making a total of 10 hookworms passed.

These dogs, with the exception of the one (No. 658) which had died, were killed on the sixtieth day and examined for worms remaining. The findings for all dogs were as follows:

Dog No. 654: 314 whipworms, 20 hookworms, 74 *Dipylidium* sp. and 1 *Taenia* sp.

Dog No. 656: 2 whipworms, 18 hookworms, and 3 *Taenia* sp.

Dog. No. 658: 3 whipworms, 5 hookworms, and numerous *Dipylidium* sp.

Dog No. 659: 34 whipworms, 242 hookworms, and 3 *Dipylidium* sp.

From the number of whipworms passed during the course of the experiment it might be suspected that onions in the diet exerted an unfavorable

effect on these worms. On theoretical grounds this might be expected, if one assumes that the position of the whipworm in the cecum is such as to expose it to anthelmintic effects much less than in the cases of worms in the stomach and small intestines. We may assume that whipworms have little resistance to anthelmintics, as Hall has noted in a number of papers, since they are occasionally removed by relatively feeble anthelmintics if these drugs enter the cecum, assuming that their removal is due to establishing contact between the anthelmintic and the worms, and this low resistance may be correlated with the fact that many substances taken in by mouth are absorbed before reaching the ileo-colic or ileo-cecal valve, may be greatly diluted before they enter the cecum, or may pass into the colon without entering the cecum if they do reach the valve, in this way failing to establish contact or to establish it in adequate anthelmintic concentration. To arrive at more definite conclusions in regard to the effect of the onions on whipworms, one must compare the number passed with the number present post-mortem.

Dog. No. 654 passed 25 whipworms and had 314 post-mortem; passed 7 per cent.

Dog. No. 656 passed 9 whipworms and had 2 post-mortem; passed 82 per cent.

Dog No. 658 passed 0 whipworms and had 3 post-mortem on the thirty-seventh day; passed 0 per cent.

Dog No. 659 passed 96 whipworms and had 34 post-mortem; passed 74 per cent.

DISCUSSION

The figures show that these dogs passed 0, 7, 74 and 82 per cent of their whipworms while on an onion diet for 37 days in the case of 1 dog and 60 days in the case of 3 dogs, the lowest figure being for the dog on the diet for 37 days. In a general way one must conclude from these figures that a substance fed in such large amounts and for a period long enough to insure its entry into the cecum on many occasions, and then removes only 7 per cent of the worms in 60 days in the case of one dog, is not a very useful or effective anthelmintic. The dogs passed 130 out of 483 whipworms, or about 27.5 per cent.

Two other factors must be taken into consideration. One factor is that of possible mechanical anthelmintic action in one case. In the case of dog No. 659 which passed 74 per cent of its whipworms, the feces frequently contained large amounts of straw and hair and the passage of this coarse material was commonly accompanied by the passage of whipworms. The writers have often noted in fecal examination of dogs that whipworms commonly come away in the presence of hair, straw, excelsior and similar objects which appear to act as mechanical anthelmintics. Hall (*4*) has noted the passage of whipworms in connection with the passage of bots fed to dogs and believed that the bots acted as mechanical anthelmintics. From the fact that whipworms are apparently never found normally detached in the intestine the writers believe that these worms attach at a given point in their early development and never change this location. The larvae apparently penetrate the mucosa by means of a buccal lancet not present in the adult. If a mechanical anthelmintic detaches a whipworm it can not reattach; a whipworm detached is a whipworm lost. Dog No. 659 showed a pronounced pica, indicated by the presence of straw and hair in the feces and in the digestive tract post-mortem. This may be correlated more or less definitely with the heavy hookworm infestation, as pica is reported for human hookworm cases, notably in the case of the clay-eaters of the southern United States.

The other factor which must be considered is the possibility that the worms passed represented in part individuals which had lived out their lives and were coming away spontaneously and without regard to the onions or other possible anthelmintic factors. Parasitic worms in the digestive tract appear to live a year or less in many cases. Post-mortem examinations of many animals the world over has indicated that in the case of many worm species the number of worms increases in the late spring and through the summer, that the infestation may continue or increase into the fall, but that there is commonly a drop in the number present in late winter and early spring. In a very comprehensive study of tapeworm incidence in 2,012 horses, Stroh (*12*) found a seasonal incidence; thus *Anoplocephala perfoliata* was least abundant in spring and became increasingly abundant in the fall. It is well known that stomach worms in sheep become troublesome in the late spring and in the summer, but are much less troublesome in the fall and winter, and that sheep killed in the late winter and early spring will commonly show a much decreased infestation. The onion-feeding

experiment discussed here was conducted from February 28 to April 29, a period during which short-lived worms might be expected to pass out. The writers reserve judgment as to the fact in this case. Apparently little is known as to the length of life of whipworms. In anthelmintic experiments for the past 10 years the writers have not observed what they regard as the spontaneous passage of whipworms, but as they have rarely carried on experiments involving the examination of the feces of an animal for two months they have had little occasion to make observations on this point. Had they carried along some control animals in connection with this experiment more definite evidence on this point might have been secured. A microscopic examination of the worms passed during the last two weeks of the experiment showed a large percentage of males, and as males commonly do not live as long as females this finding suggested that these worms were passed spontaneously; of the female worms some were gravid females containing shelled eggs and others old females with no shelled eggs, the latter finding again suggesting that these worms had died of old age and were coming away spontaneously.

The case for hookworms is as follows:

Dog No. 654 passed 1 hookworm and had 20 post-mortem; passed 5 per cent.

Dog No. 656 passed 2 hookworms and had 18 post-mortem; passed 10 per cent.

Dog No. 658 passed 0 hookworm and had 5 post-mortem; passed 0 per cent.

Dog No. 659 passed 10 hookworms and had 242 post-mortem; passed 4 per cent.

These figures, 0, 4, 5, and 10 per cent, show the onions to have had little or no effect on hookworms. A microscopic examination of 3 hookworms passed during the last two weeks showed that 2 of them were males and 1 an old eggless female, all of which points to the probable spontaneous passage of these worms as a result of old age. The dogs passed 13 out of 298 whipworms, or about 4 per cent.

The case for ascarids is as follows: Dog No. 658 passed 2 ascarids and had none post-mortem; dog No. 659 passed 1 ascarid and had none post-mortem; no other dogs were infested with ascarids. The ascarids present came away on the fifth, ninth, and either thirty-second or thirty-third day. Owing to the light infestation here one can not draw any positive conclusion. One ascarid was an adult female, another a young ascarid; no careful examination of the third was made. One would be inclined to say that if onions were anthelmintic the human ascarid should have died out among certain races and nations where it has persisted, but on the other hand it might be urged that the human ascarid has become accustomed to and tolerant to onions, whereas the dog ascarid has had no occasion to develop such tolerance and might be susceptible to certain anthelmintic effects. The writers believe, however, that if feeding onions for 32 days is necessary to remove ascarids, the onion has too little anthelmintic value to warrant its use in view of the knowledge of better anthelmintics which would remove ascarids in less than that many hours.

So far as tapeworms are concerned, it is sufficient to say that three of the dogs had an infestation with *Dipylidium* sp. and two with *Taenia* sp., that the dogs continued to pass gravid segments during the course of the experiment, and that no tapeworms came away as a result of the onion diet.

SUMMARY AND CONCLUSIONS

Raw onions in amounts of 2 oz. daily to each of 3 dogs for 60 days and to 1 dog for 37 days showed too little anthelmintic value to warrant the use of onions as a control measure for worms in dogs. The writers are, therefore, unable to urge that: An onion a day keeps the worms away.

The use of onions was evidently of no value in removing hookworms or tapeworms. In spite of the passage of numerous whipworms, the nature of some of the worms passed and the factor of mechanical anthelmintic action in the case of the dog passing 74 per cent of its worms, suggests that the worms were passed partly as the result of their death from old age and partly from the action of mechanical anthelmintics. This idea is supported by the small percentage of whipworms passed by one dog and the failure of another dog to pass any. As regards ascarids it must be admitted that all the worms present (5) were passed during the course of the experiment, but the time required, up to 32 or 33 days, to remove an ascarid from one dog indicates that there is too little merit in the onion as an anthelmintic for dog ascarids to warrant its use.

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STUDY OF THE LIFE HISTORY AND ECOLOGIC RELATIONS OF THE SMUT OF MAIZE [1]

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INTRODUCTION

The smut of maize (*Ustilago zeae* (Beckm.) Unger) has proved much more difficult to control than any of the other cereal smuts. Although Arthur and Stuart (*2*)[3] believed they had obtained control by fungicidal sprays, it has been generally accepted that there is no known practicable method of control. Neverthelesss, the current conception of the life history of the causal organism, drawn principally from Brefeld's classic investigations (*3, 4*), lends support to the idea that spraying might prevent the aerial conidia from producing infection. For this reason, further investigation of the ecology of the parasite, particularly with reference to the physiology and morphology of the host, has seemed necessary in an effort to develop methods of control. The investigations here reported cover the period from 1910 to 1920. Two brief abstracts have previously been published (*14, 18*).

Referring to experiments conducted in Kansas in 1896, Hitchcock and Norton (*8*) state, "the percentage of corn smut is here seen to rise to 26 per cent. This is, however, unusual; 6 per cent probably will represent the average." Selby and Hickman (*19*) reported as high as 25 to 45 per cent smut in limited areas of a cornfield in Ohio, but gave 4.4 per cent as the average infection. Pammel and Stewart (*15*) state that in Iowa only a fraction of 1 per cent of damage occurs for the entire state. In many Kansas fields, at the present time it is common to find 30 or 40 per cent of the plants affected with smut, while in the semiarid regions 60 to 80 per cent are frequently noted. These percentages are based on plainly visible nodal, sheath, ear, and tassel infections, and do not include the less important galls on the leaf blades. A few years ago a field was found in Nebraska which showed virtually every plant affected. Difficulties have been encountered by investigators in recording observations on corn-smut infection in percentages. It is apparent that the less conspicuous galls on the leaves and sheaths of the corn plant have been overlooked or disregarded by some, while other investigators may have included such infections in their reports. However, there appears to be sufficient reason for the belief that in the western part of the Corn Belt, at least, the disease has become much more prevalent during the last several decades.

NATURE OF INFECTION

Early observations made by the writers gave support to the statement of Pammel and Stewart (*15*) that the "lower nodes" are particularly subject to the disease and that on the same culm "where one smut boil made its appearance on the lower nodes, others appeared further up." A fair example of the data obtained on this point involves notes taken on 3,500 plants at Mitchell, Nebr., in 1914. Twenty-six per cent of these plants showed infection. This infection represented approximately 4 per cent of the buds on 3,500 plants, or 15 per cent of the buds on the infected plants.[4] Of this 15 per cent, 872 buds, or about 60 per cent of the infection, occurred on 372 plants, or 40 per cent of all plants showing any infection. In other words, 60 per cent of the total infection occurred on a little over 10 per cent of the total

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[2] The authors wish to express their appreciation for the assistance given by Ira Flemming and Ivan A. White during the course of these experiments.

[3] Reference is made by number (italic) to "Literature cited," p. 173.

[4] Counts were made to include 10 buds per plant, beginning at the bottom and counting upward to a point one bud above the ear.

number of the smutted plants, while the other 40 per cent occurred on 15 per cent of the smutted plants.

This tendency of infection to develop at more than one point on the same plant has been noted in numerous other instances (pl. 1). In 1913 a series of observations in eastern Kansas and Nebraska (and also at one point in Illinois) showed over 25 per cent of 581 infected plants to be smutted at more than one node. In the neighborhood of Washington, D. C., nearly 20 per cent of 78 plants were thus affected. In a field at Newton, Kans., in 1914, a very dry season, 15 per cent of the plants were infected and 36 per cent of the 95 infected plants were smutted at more than one node. At McPherson, Kans., with scarcely 5 per cent infection, about one-third of the smutted plants were similarly affected. At La Fox, Ill., of 58 smutted plants, 23 showed infection at two or more nodes. Again, in 1915, out of 324 smutted plants observed at points in Kansas and Indiana, over 30 per cent produced galls at more than one node. In addition to these, many hundreds of plants have been examined in the experimental plantings at Manhattan, Kans., with similar results.

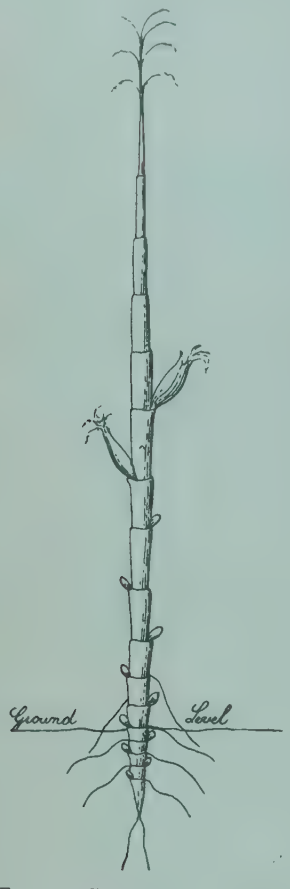


FIG. 1.—Schematic drawing of mature culm of maize, showing only those structures essential to an understanding of the presence of meristematic tissues. The apical "bud" bears several nodes with the staminate inflorescence at the tip. At about the fifth node down, the first lateral bud occurs, usually producing the pistillate inflorescence, or ear. Buds below this also may produce ears, depending on varietal characteristics and growth conditions. Toward the base of the plant the internodes are very short and buds beneath the ground level tend to develop into suckers, reproducing the structure of the parent culm. The lower buds may become infected and are sometimes mistaken for diseased "brace" roots, which are not often actually involved

Such data seemed inconsistent with the apparently well established ideas that corn smut develops its sorus only at the point of host contact and that infection arises from the mere chance of contact between aerial conidia and host plant. The fact that isolated nodal-bud infections are not entirely characteristic perhaps is not so easily explained unless it be supposed that the disease, in part at least, is systemic in character, as Pammel and Stewart (*15*) doubtless believed. Studies by the authors were begun, therefore, with the idea of checking all possible explanations of the problem presented.

THEORY OF SYSTEMIC INFECTION

The published data on the nature of corn-smut infection do not show an entirely clear conception of the development and morphology of the corn plant with relation to the occurrence of susceptible meristematic tissue. As shown by Brefeld (*4*), and more recently confirmed by Piemeisel (*16*), Pammel and Stewart (*15*) state that "the tendency to form smut boils increases in the lower nodes." Hitchcock and Norton (*8*) vaguely distinguish between infection of "rudimentary ears" and "nodes" but offer no further explanation. The number of nodal infections recorded in their table suggests that, for the most part, they should have been classified as rudimentary ears, because smutting of the true node itself is almost as rare as smutting of the internode. Arthur and Stuart (*2*) also appear to have overlooked the essential point, namely, that a bud exists at each of the lower nodes of the plant from the ear node down to the crown where the internodes become very short. Their division of the plant into "five aerial regions" is purely arbitrary. They illustrate (pl. 10, A) and record in Tables 49 to 52 the exceedingly common infection of these nodal buds as infections of the stalk or stem. MacMillan (*13*) apparently attributes the axillary infection to the presence of moisture in the sheaths rather than to the presence of meristem at the node.

MORPHOLOGY OF THE HOST

Because of the confusion which exists concerning the relation of smut infection to the structure of the corn plant, it seemed essential to clarify the conception of the morphology of the maize plant before attempting to analyze the problems of the etiology

of the disease or the relation of growth factors to the organism (fig. 1).

From the data published by Pammel and Stewart (*15*), Hitchcock and Norton (*8*, Table 3), and Arthur and Stuart (*2*, Tables 49 to 52, inclusive), as well as from the studies made by the writers, it is evident that the lower nodal buds or rudimentary pistillate inflorescences (ears) (pl. 1, D), are the most common points of infection. The foregoing additional evidence of a tendency for more than one of these buds to become infected on the same culm emphasizes the possibility of a systemic development of the disease under field conditions, despite the repeated evidence of inoculation experiments and the commonly accepted belief that the infection is strictly local in its development.

To begin with, the problem involves the possibility that if several sori appear on a plant they may have had a common origin. In this connection it should be recalled that only a comparatively short time, perhaps six to eight weeks, is required by the maize plant to develop from the stage in which the stem (morphologically speaking) is but a cone of tissue from 1 to 2 inches in length and largely in a meristematic condition, to that in which the various parts of the mature plant are differentiated and the stem has attained virtually its full height.

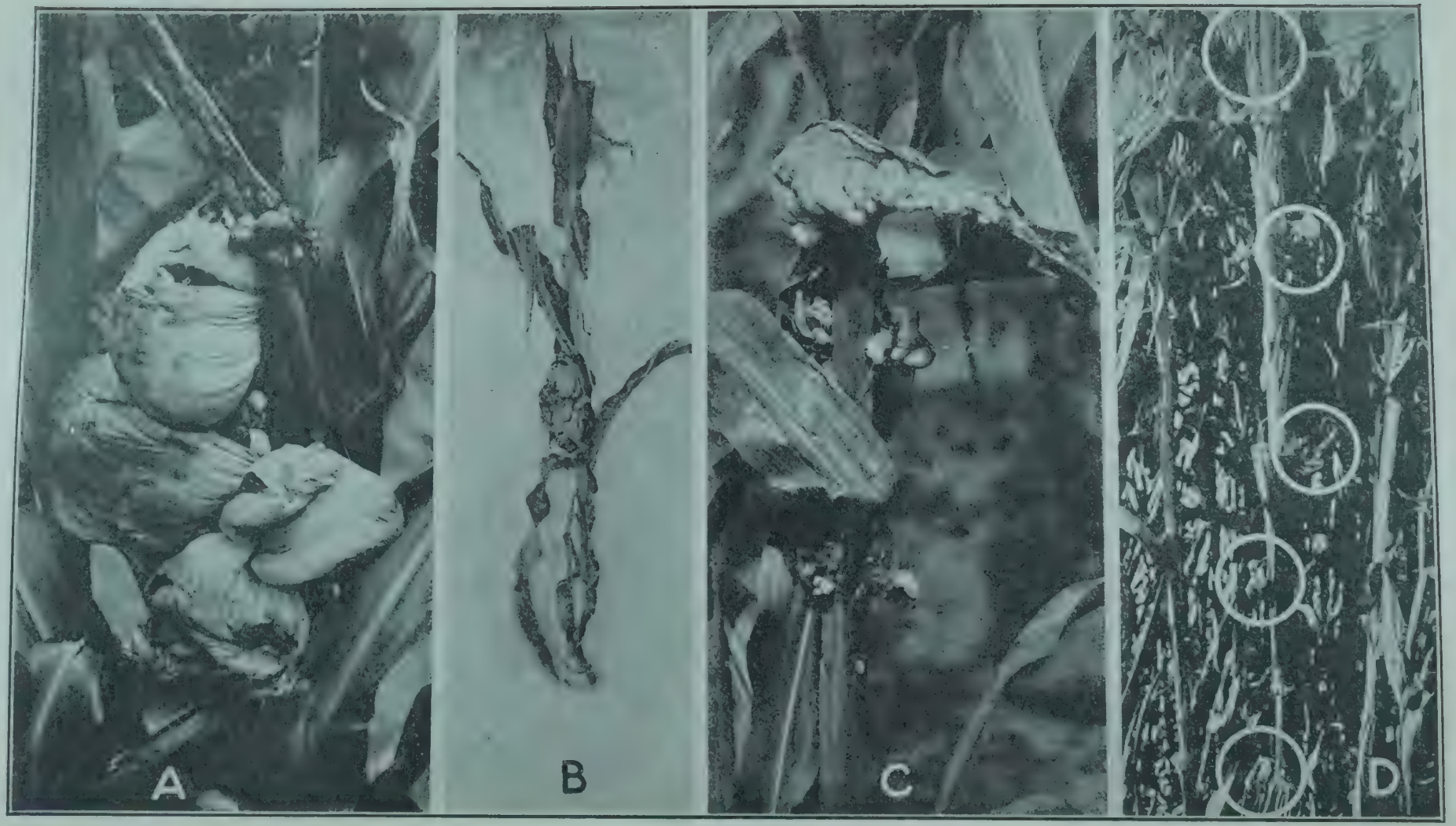
It need not be assumed, therefore, that because a plant showed several points of infection at the end of this period of rapid growth, these arose either from the growth of the parasitic hyphae from one point to another, or entirely from separate points of infection. It should be remembered that the incubation period for the smut organism in the field is generally 6 to 14 days, depending on environmental conditions. Therefore, full development and maturity of the sori would cover quite as much time as that required by the plant in growing from 1 to 2 feet in height. If, indeed, there had been but one primary infection to produce the several smut galls, it would have occurred at a time when there was but a small cone of meristem to be infected. The hyphae would have to spread only a very short distance in order to accomplish an infection in parts destined to be widely separated in the mature plant.

Brefeld has shown that corn smut may produce sori in any young tissue and that the organism does not spread from the point of infection. But neither do any of the admittedly systemic cereal smuts spread through the host tissues to any great extent. The distribution of the parasite in all such instances is brought about by the host itself when the culm or culms develop. The problem in the present instance, therefore, is to determine the reaction between the host and parasite, rather than the capacity of the parasite to penetrate the developing tissues of the host.

INFECTION STUDIES

That the relation between the host and parasite under field conditions is not one of true systemic development of the disease has become convincingly evident from the data of Pammel and Stewart (*15*), Hitchcock and Norton (*8*), Arthur and Stuart (*2*), Piemeisel (*16*), and several others, all of whom confirm Brefeld's original conclusions. The writers also have repeated extensively similar experiments with numerous variations based on the idea that *Ustilago zeae* might be found to resemble somewhat the head smut of sorghum, *Sorosporium reilianum* (Kühn.) McAlp. (*17*), which attacks both sorghum and maize (*11*, *12*). This species develops systemically in sorghum from an infection occurring later than the very early seedling stage. In this respect it resembles the "Triebinfektion" of *Ustilago violacea* (Pers.) Fckl., as noted by Hecke (*6*). However, all attempts thus far have failed to produce any evidence of systemic infection in corn smut.

Similarly, efforts to protect seedlings and young corn plants from infection have not succeeded in altering the frequency or nature of infection of plants in the field. This work has involved both chemical and thermal disinfection methods for seed and soil, as well as attempts to protect young plants from infection by careful disinfection of the seed, sterilizing the soil, and starting growth in the laboratory and in cloth-covered beds. Notes were taken on about 9,000 plants, including controls, at Manhattan, Kans., St. Paul, Minn., and Mitchell, Nebr. The lack of satisfactory methods for absolutely excluding infection and controlling other conditions, however, marred the conclusiveness of the negative results obtained. Nevertheless, the data are in accord with the results of other investigators, in leading to the conclusion that true systematic infection is not involved.



(For explanatory legend see page 165)

RELATION OF WEATHER CONDITIONS TO INFECTION

Inasmuch as the results of seed and soil treatments and inoculations have disclosed nothing to indicate true systematic development of the disease under field conditions, a more intimate understanding of the relation of weather conditions to the smut fungus and its ability to infect its host seemed necessary to an interpretation and explanation of the characteristic nodal-bud infection.

Arthur and Stuart (*2*) have elaborated meteorological observations covering a season in an attempt to demonstrate the close relationship of humid conditions to infection by the corn-smut fungus in the field. Although Piemiesel (*16*) has shown that the conidia are very resistant to desiccation, he seems to have accepted the conception of the dependence of infection on high humidities. Similarly, MacMillan (*13*) emphasizes moisture as a possible limiting factor in arid regions.

These prevailing ideas are not in accord with the fact that the disease is not peculiarly prevalent in humid areas and under humid conditions. According to the writers' observations corn smut appears to be far more abundant and destructive in the extreme western portion of the Corn Belt, in the dry hot portions of the plains of Kansas and Nebraska, than it is in the humid districts of the Eastern States. Selby and Hickman (*19*) also have observed that "more smut is found in dry seasons than in wet seasons; the same appears to hold as to situations." While this fact does not obviate the necessary relation of moisture to infection, it does appear to minimize its importance as a limiting factor in the distribution and prevalence of the parasite under field conditions.

The observations of Hitchcock and Norton (*8*) on ecological relations seem the most adequate thus far offered. They conclude, as a result of their experiments, that infection "does not depend so much on the time of the season as on the stage of development of the plant." They point out that in seasonal succession the leaves, tassels, ears, and lower nodal buds become the principal areas for smut infection, because in the development of the plant they are successively exposed in the meristematic condition. If, then, there are successive "outbreaks" of the disease, as observed by Arthur and Stuart (*2*), and more recently by Piemeisel, (*16*), it would, perhaps, be more logical to attribute these to the effect of such conditions upon the development of the host rather than to any effect upon the parasite, or, perhaps, merely to the coincidence of the condition of the host and the "frequent rains and cooler weather" of late summer (*16*). Such a season came under observation in Kansas in 1920 when the cooler weather and frequent rains in August had a stimulating effect on the growth of the corn crop accompanied by an apparent late infection of smut.

To summarize, it appears exceedingly doubtful, particularly as to infection in the ears or nodal buds, whether moisture can be considered in any material degree a limiting factor in smut development under such climatic conditions as are required for the maturity of maize.

ECOLOGIC STUDIES

Under conditions generally suited to maize culture, mainly at Manhattan, Kans., the writers began a series of investigations to determine the presence of the smut organism on the maize plant or in its environment, previous to the development or appearance of the smut. These were supplemented by attempts to shield the plants from infection, including the seed and soil treatments and the fungicidal sprays herein described.

Efforts to isolate cultures of *Ustilago zeae* from the cornfield, prior to the earliest sporulation, naturally assumed three different aspects, namely, cultures from the soil, from the air, and from the plants themselves. (See Table I.)

In order to bring the nature of this work more clearly before the reader, the history of several individual isolations (cultures) which proved virulent in the production of the disease are traced out in all the details of manipulation.

EXPLANATORY LEGEND FOR PLATE 1

A.—A plant inoculated with hypodermic needle on June 7, 1918, using culture No. 6c, isolated in 1917 and carried over on carrot agar until 1918. Smut galls evident June 18, 1918.

B.—A plant infected from the same culture, showing large smut gall at point of inoculation.

C.—A plant inoculated by means of a hypodermic needle, June 22, 1918, with culture No. 79 carried over from 1917. Smut galls evident July 2, 1918.

D.—Pseudosystemic (nodal bud) infection developed on a culm of pod corn (*Zeae maydis* var. *tunicata*). Some of the (nodal bud) infections do not show in the photograph. Note that neighboring stalks are smut-free.

TABLE I.—*Isolation and infection results obtained from several collections of the corn smut organism, Ustilago zeae, in experiments conducted at Manhattan, Kans., 1916 to 1919, inclusive*

Year	Collections			Inoculations			
	Source	Total number	*U. zeae* in culture plate [a]	Number of isolations (cultures) used	Number of trials	Infections produced	Cultures found virulent
1916	Soil [b]	15	1	Lost.			
1916	Air	43	24	3	6	1(?)	1(?)
1917	do	73	18	2	10	1	1
1918	do	301	87	44	165	14	12
1919	do	150	58	32	369	70	24
1916	Plant leaf axil	71	37	5	27	9	1
1917	do	84	39	13	120	11	6
1918	do	162	53	111	369	41	37
1919	do	103	25	20	213	15	8

[a] Some of these microscopic identifications are necessarily uncertain, because of the occurrence of yeast-like cultures and their similarity to those of the smut fungus and the impossibility of isolation for adequate study because of contaminations.

[b] Isolations from soil proved very difficult because of the large number of other organisms capable of more rapid growth. In more recent work isolations from the soil have been successful.

Culture No. L 44c was isolated on June 14, 1916, when the plant was about 1 foot high. With a specially made sterile pipette, about 1 cc. of liquid was drawn from the axil of one of the upper leaves of plant No. 44 in row "L" of the experimental plat.

Immediately after drawing the liquid from the leaf axil of a plant it was placed in a sterilized tube, taken to the laboratory, and plated out in carrot agar. These plates were kept at from 20° to 23° C. Five days later, three colonies of *Ustilago zeae* (designated hereafter as L 44a, L 44b, and L 44c) were noted in the plate in the midst of other fungous and bacterial growth. These colonies had been identified a few days earlier under the microscope (low magnification) by the characteristic "snowflake" appearance. Plate 3, A and B, shows a photomicrograph of two colonies from culture plate No. L 44c, with similar colonies, C and D, from a known culture of corn smut for comparison. Because of the slow development of the smut colonies as compared with the contaminations in the culture plate, it was necessary to remove them as soon as possible. This was done by ringing the colonies, waiting a few days until they became visible to the naked eye (about 0.1 to 0.2 mm. in diameter), and then removing the colony by means of a flat platinum wire (*9*), taking pains to leave a bit of the agar surrounding the fungous growth.

The characteristic gray-white color and "stringy" consistency of the fungous mass in the culture medium in the early stages of growth made it possible in subsequent studies to isolate the colonies without preliminary microscopic identification (pl. 3, D). It was found also that contamination by bacteria could be largely eliminated by adding two ot three drops of a 5 per cent solution of lactic acid to 10 cc. of the carrot agar. This did not inhibit the growth of the smut fungus.

Cultures of *Ustilago zeae* show a greater tendency to hyphal or mold-like growth than do those of head smut of maize and sorghum, *Sorosporium reilianum*. In old cultures this frequently appears as a whitish pubescence over the surface of the growth, or the white hyphæ are noticeable at the margins of the cultures. This characteristic does not seem to be dependent entirely upon the age of the culture after transfer but, to some extent, at least, upon its age from the time of spore germination. This has been most commonly noted on carrot agar. Generally after transfer the colony darkens gradually through tan, brown, or olive, unt l almost black, the exact appearance being affected by the hyphal development which seems inclined to occur in spots over the usually rugose surface of the growth. In old cultures the consistency becomes almost leathery; somewhat like the fruiting body in the Hymenomycetes.

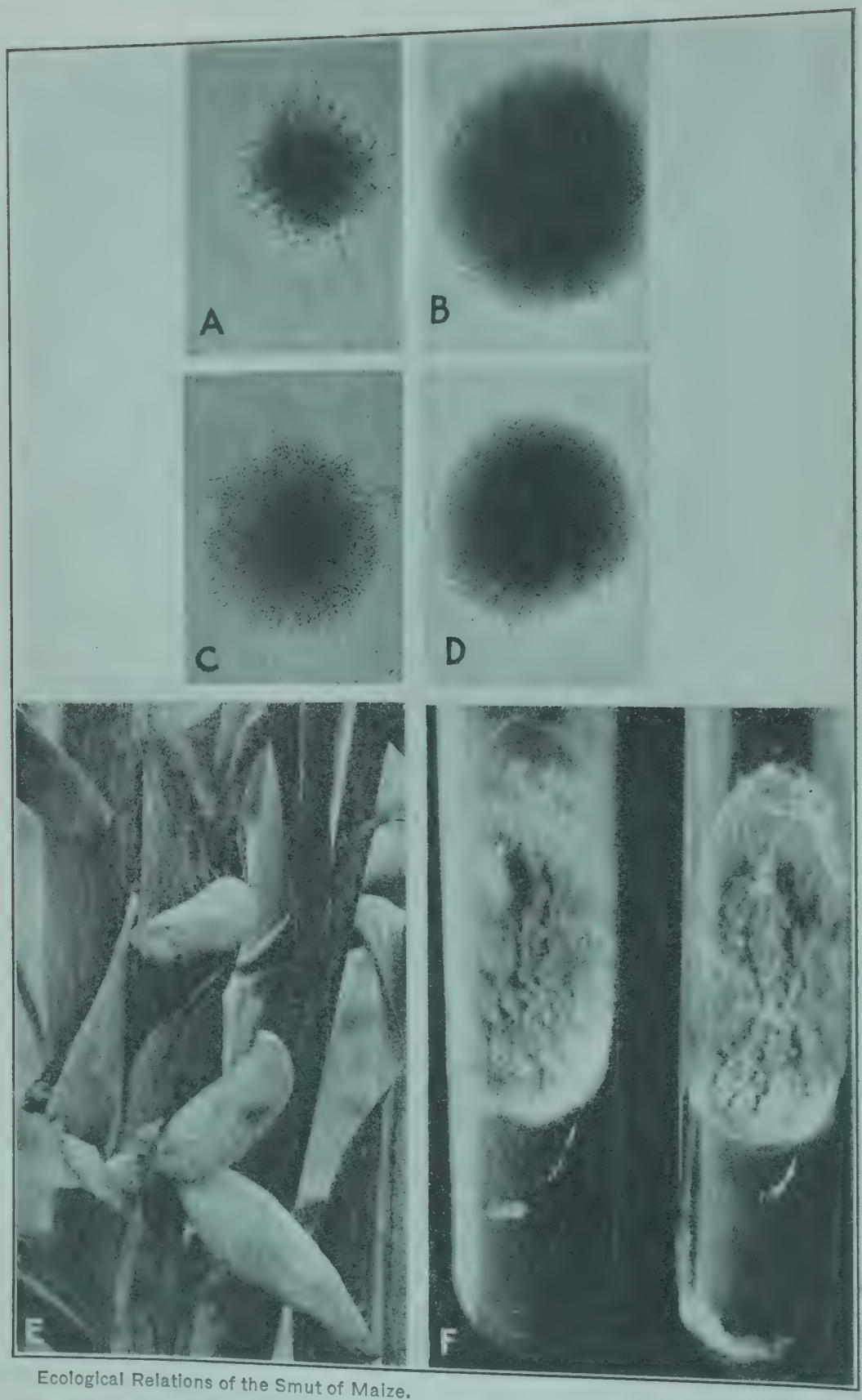
Having determined by the cultural characteristics above described that the three isolations, a, b, and c, obtained from the plating of No. L 44, corresponded to cultures of corn smut, an effort was made to verify this identification by using them in inoculations. Transfers were made to tubes

Ecological Relations of the Smut of Maize. Plate 2

Culture L 44c was used in producing these infections.

A.—Infection produced by allowing a mixture of 2 cc. culture decoction and 5 cc. distilled water to trickle down into the leaf axils. This was poured into the top of the plant. Not all of the smut lesions are shown in the illustration; arrows indicate visible sori.

B.—Infection resulting from injection of liquid culture by means of a hypodermic needle.



Ecological Relations of the Smut of Maize.

Plate 3

Figs. A, B, C, and D, photomicrographs of colonies of *Ustilago zeae* on carrot agar plates, two days old from conidial sowing at 20° to 22° C. × about 100.

A.—Small colony, isolation L 44c.

B.—Large colony, isolation L 44c.

C.—Small colony, single spore (chlamydospore) isolation of *Ustilago zeae* for comparison with A.

D.—Large colony of *U. zeae* (from chlamydospore) for comparison with B.

Figs. E and F.—Photographs about one-half natural size.

E.—Plant inoculated with culture No. 88, in the same manner as described for cultures Nos. 6c and 79 in legend of Plate 4.

F.—Cultures of *U. zeae*. Note the rugose surface.

of carrot decoction which were allowed to incubate at 23° to 25° C. for two or three days, until the culture appeared turbid and sometimes covered with a thin scum of aerial conidia. These liquid cultures were used to inoculate young plants either by pouring them into the leaf axils, or by injecting them into the region of the growing point (6 or 10 inches from the ground in plants 12 to 18 inches high) by the use of a hypodermic needle. Of the three isolations, all of which were used in numerous inoculations, No. L 44c alone gave positive results, producing smut in almost every trial. Plate 2 well illustrates its virulence.

Isolations Nos. 6c, 79, and 88 were made in 1917 and may be taken as typical examples of virulent cultures. Continuously growing them on artificial media did not seem to reduce their virulence when used for inoculation.

Culture No. 6c was isolated as described for No. L 44c, from the topmost leaf axil of a plant 5 inches tall. Three isolations taken from this same agar plate proved virulent. The results of an inoculation with "6c" are shown in Plate 7, A, B, and C.

Culture No. 79 was isolated by using photographer's porcelain developing trays containing a small quantity of distilled water and having a surface 5½ by 8 inches. These were exposed for 16 hours in the cornfield. Of the 12 cc. of liquid recovered from the water plate, one-third was used in pouring the agar plate for isolations. Numerous colonies appeared in the plate. One was isolated and proved virulent in a number of trials (pl. 7, C).

Culture No. 88 was isolated the latter part of July from the axil of the ninth leaf of a fully developed corn plant. This proved virulent in several trials in both 1917 and 1918 (pl. 3, E).

From Table I, it will be noted that the isolation of cultures which seemed to be morphologically identical with those of *Ustilago zeae* was accomplished frequently and with little difficulty. In many cases, however, there remained some doubt as to the identity of such cultures, because the smuts in artificial culture or as young colonies closely resemble numerous yeasts and other somewhat similar organisms. The fact that many of the cultures proved virulently pathogenic leads to the belief that others also may have been pure cultures of *U. zeae*, although no infections were obtained. Such an assumption is supported by the coincident failure of virulence in known cultures originally made from a single chlamydospore of *U. zeae*. The plants used for inoculation were grown from seed of open-pollinated ears. This undoubtedly could explain part of the irregularities obtained in such inoculations, and is supported by results of experiments now being conducted in Kansas to determine relative resistance of inbred strains of corn to smut.

The nature of the infection, also is pertinent in such cases as those noted by Pammel and Stewart if we may but alter their statement to read, "where one smut boil made its appearance on the *upper* nodes others appeared farther *down*." In other words, it would seem that such infections frequently arise from one and the same contamination of axillary moisture by *Ustilago zeae* at a relatively early period in the plant's development. This axillary culture then spreads by running down into the lower axils. This view of axillary contamination has appealed also to Arthur and Stuart (*2*), except that they do not trace the several galls to the same contamination, nor have they evidence that *U. zeae* ever was present in the leaf axils. The writers have produced such infections by artificial inoculation, an example of which is seen in Plate 2, A.

At the beginning of these investigations the point which appealed to the writers as most pertinent to the problem of fungicidal control was the probability that aerial conidia falling upon the corn plant produce direct local infection only in some instances. Susceptible tissues of the host are seldom exposed to direct attack, except perhaps by some injury (*7*), so that in the normal course of events an interval must occur between the contact of the conidium with the host and actual infection of the tissues. In this respect *Ustilago zeae* may be considered as distinct from other more highly specialized parasites among the cereal smuts, most of these being capable of infecting only the very young seedlings. The latter necessarily have to enter their hosts in the seedling stage if they are to infect at all.

Corn smut, on the other hand, may be considered frequently pseudosystemic in its attack, as it appears to exist *on* the host rather than *in* it prior to the development of the disease in its most typical form in the nodal buds or ears. This fact is of interest in view of the positive statement of Arthur and Stuart (*2*) that fungicidal sprays gave definite indication of control. The writers, therefore, have attempted similar treatment with several variations, not so much with the idea of any immediate practical importance which

might result from such spraying experiments, as to obtain confirmatory evidence in the study of the ecologic factors affecting the development of the corn-smut organism. These results are briefly described below.

THE POSSIBILITIES OF CONTROL

In attempting a study of control measures, the following three general lines of procedure suggested themselves, namely, fungicidal treatments, cultural methods, and the development of resistant strains or varieties of corn.

FUNGICIDAL SPRAYS

The only experiments with fungicidal sprays which had been conducted up to 1910 when these investigations were begun, were those by Kellerman (*10*) in Kansas, and Stuart (*20*) and Arthur and Stuart (*1*) in Indiana. These early investigations, however, were not extensive and no record was made of the significance of the effect of the sprays on the corn crop, particularly the yield.

In the spraying experiments conducted by the writers, approximately 13,000 plants were used during the several seasons in which the work was done at Manhattan. (See fig. 2.) The yield of grain per plant always *increases with* the percentage of smut found in the plant. In other words, the effect of the fungicides has been to decrease not only the smut but also the yield. The figures on yield may be accepted as a measure of the general reduction in vegetative vigor due to injury done by spraying. It seems therefore, that an adequate interpretation of these data, which obviously give a distinct indication of smut control, must give due consideration to the host injury occasioned. Arthur and Stuart (*2*) noted some such injury in their experiments and, as in the work at Manhattan, were not successful in attaining complete control. Their conclusion, however, that true fungicidal control had been attained would seem hardly to be justified by the mere fact of reduction in infection percentages. Plants which have been so definitely reduced in vegetative vigor would necessarily produce less meristematic tissue, a circumstance which involves a correspondingly lessened production of smutted tissues.

In order to understand the spraying results at Manhattan, Kans., it seems sufficient to present only briefly the formulas of the sprays used. The Bordeaux mixture sprays were: 6–4–50, 4–5–50, 3–4–50, 1–1–50, and the lime-sulphur solutions were 1 part of the concentrate to 30 or 40 parts of water. Formaldehyde solutions of the strength 0.1 and 0.2 per cent were used, and during one season $CuSo_4$, one part to 100 of water, was tried.

In support of the conclusion that true fungicidal control is doubtful, may be mentioned the fact that both conidia and chlamydospores will germinate on glass slides sprayed with certain strengths of Bordeaux mixture, copper sulphate, and lime-sulphur. Similar observations have also been made by Dandeno (*5*). The writers have made no effort to analyze the many complications involved in comparing these laboratory germinations with results obtained from the use of these fungicides in the field. It is only desired to present here the observation corroborating the conclusion that it is very questionable whether real fungicidal effect has been attained in any attempts to control corn smut by spraying.

The efforts to prevent infection having yielded essentially negative results, it would appear desirable to note the points on which further research might yield results and indicate the lines of procedure for developing possible control measures. Since it has been shown that the smut fungus exists in an active virulent condition in the leaf axils of corn in the field, it appears possible that some way might be found for suppressing such virulence, even though the culture actually can not be killed without injury to the host. Piemeisel (*16*) has shown that virulence may be retained for some time in artificial culture. The experience of the writer substantiates this, but since some cultures apparently lose the power to infect, it seems more pertinent to the problem of control to discover when and why virulence is lost. If the answer to this question discloses no feature which could be put to practical use in controlling the disease, there still remains a possibility that methods of applying chemical dusts or even of other sprays may be developed which will avoid injuring the plant. This must be accomplished before satisfactory observations of control can be made, and probably will necessitate trials with other chemicals than the fungicides commonly used as liquid sprays.

DATE OF PLANTING

Studies have been made during several seasons on the effect of the date of

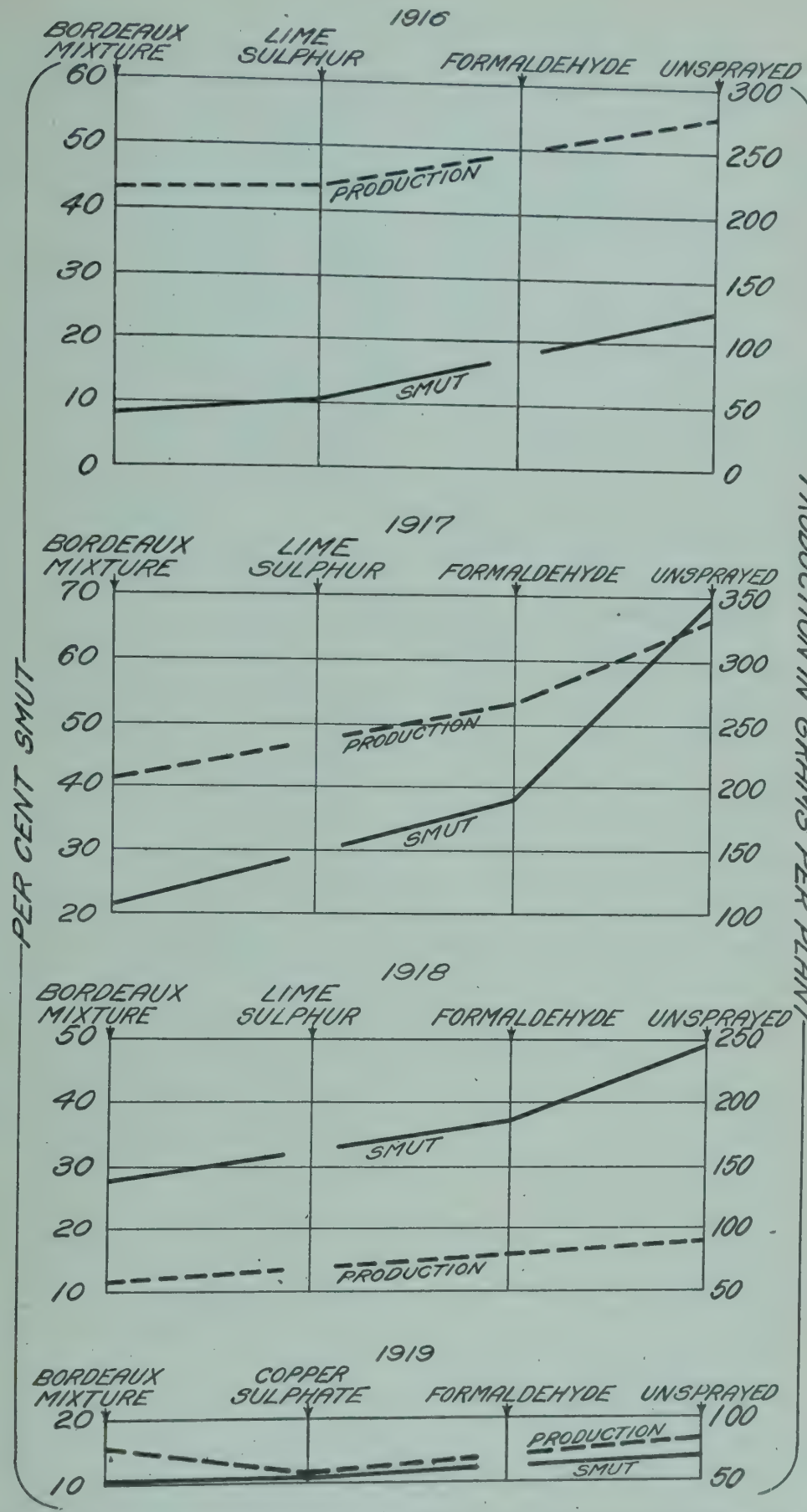


FIG. 2.—Graphic representation of production (weight of ears) per plant and percentage of smut in sprayed and unsprayed plats in four successive seasons, 1916 to 1919 inclusive, at Manhattan, Kans. Where the curves are broken at aperpendicular, it is indicated that data on that chemical were not obtained in that year. Commercial white corn was used in these experiments

planting the corn upon infection by smut. It was believed that ecologic information on the prevalence of conidial distribution of the organism might be gained by planting certain varieties at successive dates during the season. The varieties used were Kansas Sunflower, Hildreth's Yellow Dent, Boone County White, and Commercial White. They were planted, in so far as possible, on April 15, May 1 and 15, June 1 and 15, and July 1, in the years 1915 to 1918 inclusive.[5] Plantings were made at Manhattan and Hays, Kans. The results seemed to show less infection in plantings made on June 15 and July 1 than in earlier plantings. This might be due to the fact that fewer conidia occurred at the critical period of infection for these late plantings and that environmental conditions were less favorable for the development of the organism. However, the plantings made on July 1 were always only in the tasseling stage when the first killing frost occurred. This relative immaturity of the host would also affect the extent to which the plants developed visible evidence of infection. Apparently, no evidence can be adduced from these results which would warrant conclusions of ecologic value. Experiments now being conducted by the junior author, in which strains of corn homozygous for the character of resistance and susceptibility to smut are being used for dates of planting, will no doubt give data of value on this point. Observations, however (these data are summarized by years in the inoculation results in Table I), have indicated the increasing prevalence of conidia in the cornfield as the season advances. It is impossible to escape this increase by early planting because of the long growing period of the crop. The early planting of early-maturing varieties, as of sweet corn, might prove of some value.

VARIETAL RESISTANCE

Experiments to discover possible varietal resistance to corn smut were made with 25 varieties of maize in the three years 1916 to 1918 inclusive. Negative results were obtained. None of the varieties showed any consistent behavior relative to the development of smut infection, the differences being insignificant and more or less variable. Hitchcock and Norton (*8*), after an extensive series of tests in 1894–1895, also concluded that "there is little difference in varieties of corn as to their susceptibility to smut."

Because of the heterozygous nature of the corn plant, these essentially negative results might have been anticipated. However, judging from the experiments which are being conducted at Manhattan at present, it seems possible to expect that in many varieties, inbred strains may be secured which will vary from complete susceptibility to marked resistance.

Furthermore, the problem of breeding for resistance may become still more complex if specialized forms of the organism are found to exist in different localities (*14*). In such an event, it would have a very significant bearing on the program of breeding for resistance. This phase of the work is being investigated by the junior author at the present time.

SUMMARY

(1) Corn smut is widespread throughout the Corn Belt and is becoming an important limiting factor in corn production in regions of relatively scant rainfall. Owing to the nature of corn smut infection it has been found impossible to control the disease by methods commonly applied to other cereal smuts.

(2) A clear conception of the development and morphology of the maize plant with relation to the occurrence of meristem susceptible to infection, is requisite to an adequate interpretation of the field phenomena which often simulate systemic infection. The rudimentary ears of the lower nodes are most commonly involved.

(3) Neither inoculation studies nor attempts to shield young plants from infection in the field have produced evidence to indicate systemic development of *Ustilago zeae* in corn plants in the field. Earlier observations bearing on this point, therefore, are fully confirmed.

(4) An ecologic study covering four crop seasons has indicated that moisture is not a limiting or controlling factor in the occurrence of corn smut, and that infection does not depend so much on the time of the season as on the stage of development of the host plant.

(5) The aerial conidia falling upon the corn plant do not appear to produce direct local infection so frequently as is indicated by the literature on the life history of corn smut. A common method of infection is the development of a virulent culture in moisture held in the axil of a leaf of a young plant.

[5] The data at Manhattan, Kans., were obtained from the plots grown by the Department of Entomology of the Kansas Experiment Station.

Such a local culture is likely to produce other infections in adjoining nodal tissues and thus produce a pseudosystemic development of the disease.

(6) Apparent partial smut control with fungicidal sprays has been shown to be due to host injury by the spray, resulting in a reduced vegetative activity, a circumstance which involves a correspondingly lessened production of smutted tissues. Wherever the smut was lessened by fungicides, a similar reduction in yield occurred.

(7) Planting numerous varieties of corn on several successive dates in an attempt to avoid infection gave only negative results.

(8) Strains of corn can be secured by inbreeding which show great variations in susceptibility and resistance to smut. Their behavior in different regions is being studied in an attempt to discover whether a difference may exist in the corn-smut organism. This phase of the problem is now being studied at Manhattan, Kans.

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FURTHER STUDIES ON THE TOXICITY OF JUICE EXTRACTED FROM SUCCULENT ONION SCALES[1]

By J. C. WALKER, *Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture,* and CARL C. LINDEGREN and FREDA M. BACHMANN, *formerly Research Assistants, Department of Plant Pathology, University of Wisconsin*

SCOPE OF THE INVESTIGATION

In a previous paper reporting studies on the nature of resistance to onion smudge, the senior author (*7*)[2] has discussed the toxicity of volatile substances of the host to the causal fungus, *Colletotrichum circinans* (Berk.) Vogl. It was pointed out at the time that, although these substances did not apparently contribute to the conspicuous varietal resistance of highly pigmented, as compared with unpigmented, varieties of onions studied, they probably did play a rôle in restricting the parasitism of the organism. The obvious question arose during the course of these studies as to the effect of these volatile substances upon other parasites of the onion bulb. As one approaches these fundamental questions of parasitism the complexities become increasingly apparent. The interrelations with the host are probably specific for each parasite. Each invader attacks by its own method and the defense of the host may in each case be different. The present investigation was undertaken primarily with the idea of throwing further light, if possible, on the general question of parasitism.

In this work the crude mixture of volatile substances arising from onion tissues, usually after crushing, was used. It is often erroneously stated in textbooks that the chief volatile constituent of the onion is allyl sulphide $(C_3H_5)_2S$). Semmler (*4*), however, found that the volatile oil from onion contains no allyl sulphide, but is made up principally of a disulphide, $C_6H_{12}S_2$. It also contains a higher sulphide of the same radicle and another sulphur-containing compound which is probably identical with one of the higher-boiling asafetida oils. Since any attempt at isolation of the individual substances is likely to alter considerably the complex as it exists in the plant, it was considered best for the purpose of this investigation to study first their combined effect upon the fungi. In considering the results, therefore, it is evident that one or more compounds may be responsible for the toxic effects observed.

It is of interest to note that since the completion of the senior author's last paper (*7*), Brown (*2*) has published his studies on the effect of volatile substances from various plant parts upon spore germination. He notes a stimulating effect in the case of most tissues tried, but when onion tissue was used marked inhibition was found to occur. Brown also points out that in an ordinary moist chamber, formed by placing moist filter paper in the bottom of a closed dish, there is a biotic reaction which results in the fermentation of the filter paper and this process yields a volatile substance toxic to the fungi which he studied. He did not find this to occur, however, until several days after the filter paper was moistened. Since all of the writers' observations were made within 24 hours after initiation of the experiment, and since all experiments were controlled, they do not consider that this factor has entered into the results. With the method of experimentation used it was impossible to duplicate actual conditions which the parasite encounters upon entering the host tissue. It is quite probable that the volatile substances released upon crushing the tissue do not occur thus exactly as they existed in the host cell. The manner in which the fungus is exposed to the onion oil may differ widely as between the experiment and nature. Many of the bulb parasites

[1] Received for publication May 3, 1924; issued April, 1925. This study has been supported jointly by the Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, U. S. Dept. of Agriculture, and by the Department of Plant Pathology, University of Wisconsin, through a special grant from the general research fund of the University.

[2] Reference is made by number (italic) to literature cited, p. 87.

alter the host tissue in advance of the hyphae, and in that process part or all of the toxic substances may be changed or rendered inactive. These facts naturally limit the value of the experimental data in answering finally many of the questions which arise in connection with parasitism.

In order to reduce to a minimum the influence of outside factors upon the results the procedure was standardized as much as possible. This involved a study of the relation of temperature, age of spores, and age and condition of bulbs to the toxic effects of the volatile substances. Having worked out these relations primarily with a single fungus (*Colletotrichum circinans*), the comparative study of a number of fungi was begun.

METHODS

The volatile substances were obtained from onion juice which was secured by grating the succulent onion bulb tissue, placing it on a dry filter paper, and allowing it to filter without pressure. In certain cases, when a large amount of liquid was desired, the grated pulp was first placed in cheesecloth and the juice pressed out before filtering. No appreciable difference in the toxicity of extracts obtained by the two methods was noted, but to avoid any possible variation due to this cause, a single method was used throughout any given experiment.

A spore suspension of the fungus in question was made in sterile nutrient liquid, either potato or onion decoction. Drops of the spore suspension were placed on a glass slide in a moist chamber. Another slide containing one or more drops, as needed, of the expressed onion juice, was placed in the same chamber. Control chambers without onion juice were always included in the tests. In all these experiments Petri dishes of uniform size, lined with moistened filter paper, were used as moist chambers and definite amounts of expressed onion juice were used as sources of the volatile substances.

Observations were made after 6 to 24 hours, depending upon the rate of germination and growth of the fungus in question. In order to stop growth throughout a series at a given time, chromacetic killing fluid was added to the drops at the end of the period of exposure and the counts and measurements were made as soon as possible. In measuring the effect of the toxic substances upon the fungi the percentage of spores germinating and the rate of growth of the sporelings were recorded. The former was determined by counting 100 spores or more per slide. The rate of growth was estimated by measuring and averaging the length of the thalli of from 10 to 40 sporelings on each slide.

FACTORS AFFECTING THE TOXICITY OF VOLATILE SUBSTANCES FROM THE EXPRESSED JUICE

METHOD OF EXTRACTION

As previously stated, no appreciable difference was found in the extracts secured by the two methods used, namely, (1) by grating the succulent bulb tissue and allowing the liquid to filter without pressure, and (2) by extracting the juice from the grated tissue through cheesecloth and then filtering.

AGE AND CONDITION OF BULBS

Yellow Globe onions of a single lot were used in nearly all these experiments. They were secured in the autumn directly after harvest and stored in a basement the temperature of which remained at 12° C. or less during the winter months but gradually approached room temperature after April 1. The investigations extended from autumn until June. During this period a reduction in the toxicity of onion extract was observed and this became especially noticeable after March. A similar reduction was noted in 1922 in onions of the previous autumn's crop obtained in the markets at Madison, Wis. It is to be expected that metabolic changes in the onion bulb continue during the protracted storage period. This apparently brings about some reduction in the volatile toxins, especially with rise in temperature. A marked difference in this respect was also noted between sprouted and unsprouted onions during the latter part of the storage period. Juice from sprouted onions has always been found to be less toxic than that of unsprouted onions from the same lot.

AGE OF CULTURES

Early experiments in which uniform results were not always secured when spores were not taken from a common source indicated that the age of the spore affected the degree of its resistance to the volatile toxins. In order to establish the importance of this factor, the spores of both *Colletotrichum circinans* and *Botrytis allii* from potato agar cultures of different ages were compared under identical conditions. The data in Table I show

that in the controls there was a reduction in viability and vitality in spores of both species as the culture aged. 1, in which the average length of germ tubes of the different cultures is compared. It thus became evident that,

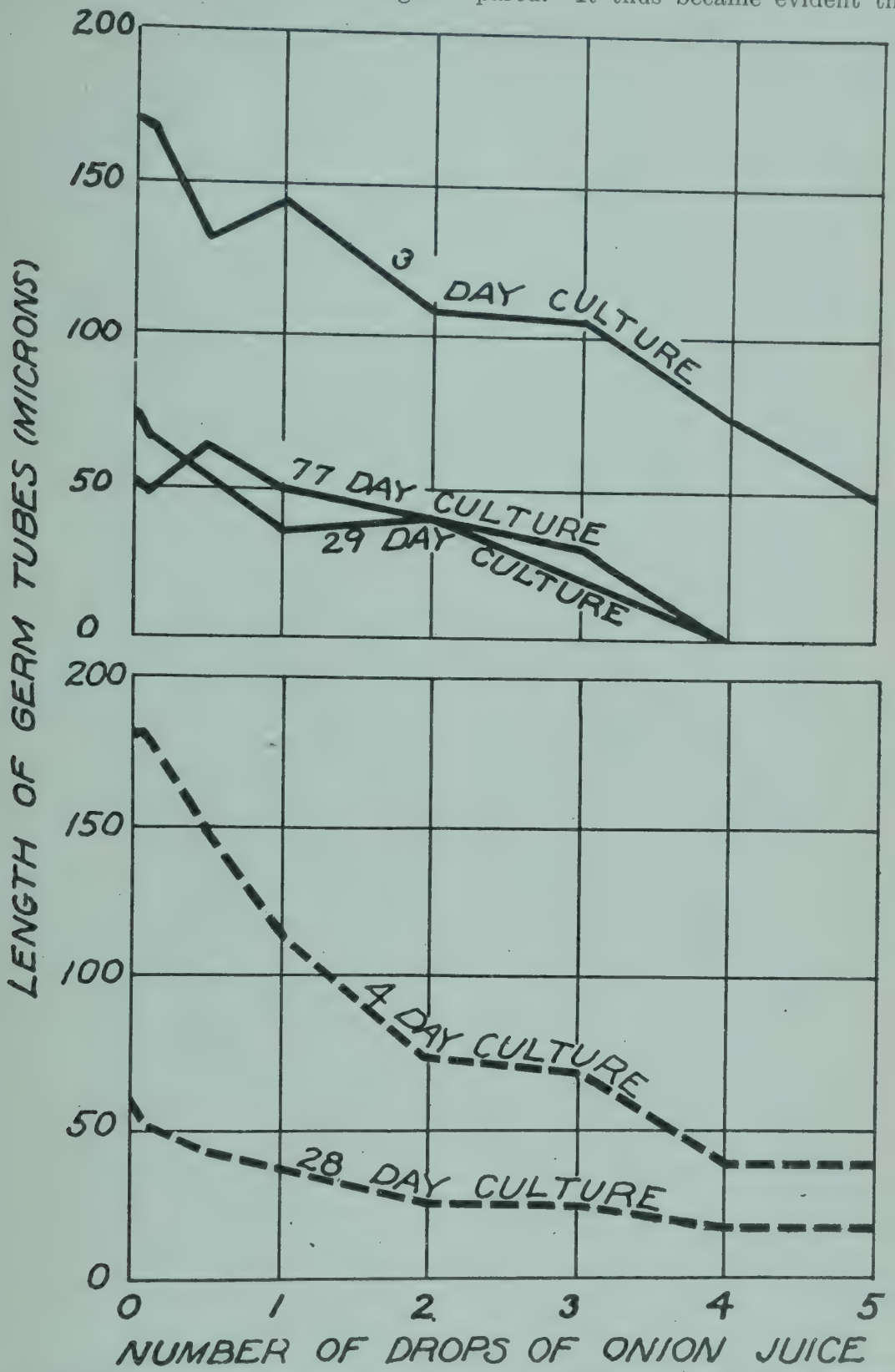

FIG. 1.—Upper graph represents the growth of sporelings of *Colletotrichum circinans* and the lower graph the growth of *Botrytis allii* from cultures of different ages, when exposed to various concentrations of volatile onion oil. See data in Table I

This reduction in vigor is correlated with a diminished resistance to the volatile toxins from the onion. This fact is especially emphasized in Figure

with a given comparative series, spores as nearly uniform in vigor as possible should be used and preferably those from very young cultures.

TABLE I.—*Comparative germination and growth rate of spores of Colletotrichum circinans and Botrytis allii of different ages in relation to the volatile substances from onion juice* [a]

Organism	Age of culture from which spores were secured		Number of drops of onion juice in chamber— 5	4	3	2	1	0.5	0.1	0.01	0.001	Control
	Days											
Colletotrichum circinans.	3	Germination (per cent)	93	88	93	93	91	94	95	91	93	95
		Length of germ tubes (microns).	49	73	106	108	144	132	167	165	164	170
	29	Germination (per cent)	0	0	2	7	98	98	96	99	100	99
		Length of germ tubes (microns).	0	0	-----	41	36	53	66	79	70	74
	77	Germination (per cent)	0	0	2	0	46	44	39	32	60	44
		Length of germ tubes (microns).	0	0	31	0	50	63	50	53	45	52
Botrytis allii-----	4	Germination (per cent)	78	71	98	96	86	92	91	86	84	97
		Length of germ tubes (microns).	39	39	69	74	115	149	180	171	191	180
	28	Germination (per cent)	49	67	63	78	85	88	92	85	79	85
		Length of germ tubes (microns).	18	18	25	26	37	42	53	50	57	60

[a] Duration of experiment, 13 hours at room temperature.

EFFECT OF HEATING THE EXTRACT

The effect of heat upon the toxicity of onion juice was studied. It was reported earlier by the senior author (*1*) that in onion juice which had been heated in live steam for 20 minutes the spores of *Colletotrichum circinans* germinated and the mycelium grew rapidly. Numerous repetitions of this experiment show that, although this sometimes occurs, in the majority of cases the extract will support neither germination nor growth, even though heated for an hour in the steamer or autoclaved for one-half hour. Brown (*2*) reports that the inhibitory effect of the volatile substances arising from onion juice is greatly reduced or destroyed by boiling.

It is to be expected that if the volatile onion oil consists of three compounds, as noted by Semmler (*4*), each with a different boiling point, the application of heat may remove or destroy certain of these more readily than others. Provided that they are all toxic, one would expect a gradual reduction in toxicity, depending on the temperature and the duration of heating. Two experiments on this phase of the problem are reported.

EXPERIMENT No. 1.—Juice of Yellow Globe onions was filtered and divided into four samples of 10 cc. each and a remainder which was kept for a control. Two samples each were heated for varying periods in a water bath held at 70° and two at 96° C., respectively. To one tube in each bath a reflux condenser was attached to reduce the loss of the volatile substances. One cubic centimeter samples were removed from each tube after 15, 30, 60, and 90 minutes. The samples thus obtained were tested by the usual method for the presence of volatile and dissolved toxins.

The data given in Table II and represented graphically in Figure 2 show that, on the whole, there is a gradual decrease in the amount of volatile toxin with continued heating and that the toxicity is reduced more rapidly at the higher temperatures. With the juices heated in open tubes there is a smooth curve produced, indicating a gradual loss in toxicity, but when the reflux condenser was attached there was a decided drop in both curves at 30 minutes, indicating greater toxicity in juice thus heated than in that heated only 15 minutes. Obviously, an explanation of this fact will depend upon further analysis of the volatile substances and their reaction to temperature.

The dissolved toxins were still sufficiently concentrated to kill any of the spores introduced directly into the extract.

EXPERIMENT No. 2.—Five cubic centimeters of onion juice was placed in a side-neck test tube and distilled over an open flame. About 1 c.c. of distillate was collected. The distillate and residue were then tested by the usual method for both dissolved and volatile toxins. (See Table III.) Although distillation was not continued for more than 5 minutes, the heating seems to have had

TABLE II.—*The effect of heat on the toxic volatile substances in onion juice as determined by their effect upon Colletotrichum circinans*

Source of volatile oils	Method of exposure of fungus	Treatment of juice: Method of heating	Treatment of juice: Temperature (°C.)	Response of fungus to exposure to juice heated for various intervals: Percentage of germination: 15 minutes	30 minutes	60 minutes	90 minutes	Average length of germ tubes (microns): 15 minutes	30 minutes	60 minutes	90 minutes	Response of fungus to unheated juice and to control medium: Germination (per cent)	Average length germ tube (microns)
Heated juice.	Five drops placed in moist chamber.	Reflux	70	99	99	99	98	52	39	69	81		
			96	100	99	99	99	76	68	108	91		
		Open heat	70	99	99	98	99	51	62	64	87		
			96	99	99	99	99	75	86	93	93		
	One drop of juice placed in one drop of onion decoction spore suspension.	Reflux	70	0	0	0	0	0	0	0	0		
			96	0	0	0	0	0	0	0	0		
		Open heat	70	0	0	0	0	0	0	0	0		
			96	0	0	0	0	0	0	0	0		
Unheated juice.	Five drops placed in moist chamber.	None										76	10
	One drop of juice placed in one drop of onion decoction spore suspension.	None										0	0
Control onion decoction spore suspension.		None										99	109

a decidedly destructive effect upon the volatile toxins. Both distillate and residue were practically devoid of volatile toxins, and the former contained a much reduced amount of dissolved toxin. It appears that the toxicity is due to at least two components, one of which is readily volatile and the other less so or not as all. The volatile toxins are largely broken down by boiling, while the toxic property of the residue is not destroyed by heat. This is in accord with the generally accepted view that onion oil is decomposed by distillation at ordinary pressure (*4*).

TEMPERATURE DURING THE TEST.

Certain irregularities in the results led the authors to suspect that the temperature during the experiments influenced the effect of the volatile substances upon the spores. Two sets of relations are to be considered: First, that of temperature to spore germination and growth; and, second, that of temperature to the volatilization of oil from the onion juice. The first set of conditions for *Colletotrichum circinans* has been studied previously by the senior author (*6*). It was shown that the rapidity of

TABLE III.—*The effect of distillation on the toxic substances in expressed onion juice, as determined by tests with spores of Colletotrichum circinans*

Method of exposure of fungus	Source of volatile oils	Response of fungus spores: Per cent germination	Average length of germ tubes (microns)
5 drops placed in moist chamber	Distillate	100	94
	Residue	100	95
	Unheated juice	76	10
1 drop of liquid added to 1 drop of onion-decoction-spore suspension.	Distillate	94	18
	Residue	0	0
	Unheated juice	0	0
Control onion decoction spore suspension		99	109

germination increases up to about 20° C. and then decreases, while growth increases to about 27°, then declines abruptly. It is to be expected that the volatilization of the oil would gradually increase with the temperature up to a much higher degree. A marked difference in the rate of depletion of volatile toxins in expressed onion juice held at high and low temperatures is shown in Table IV. Juice from a common source was divided into two lots immediately after extraction; one lot was placed in the ice box, the other in a constant temperature incubator at 25°. Tests with juice from the two lots at given intervals show that at the higher temperature the volatile toxins disappear within a very few hours and before any evidence of bacterial fermentation was noted. In the juice kept at the lower temperature the volatile toxins were still distinctly evident after 22 hours. The dissolved toxins persisted apparently until fermentation by contaminating organisms had reached an advanced state.

In view of this evidence that the rates of volatilization of the oil varies greatly from 10° to 25° C., it is to be expected that the temperature at which spore germination tests are made may influence the results. The extent of this influence was not as great as might be expected. Several experiments were conducted in which various amounts of onion juice were placed in constant temperature incubators over a range of 10° to 28°. Evidently the lowering of temperature had sufficiently similar retarding effects upon volatilization of the oils and upon

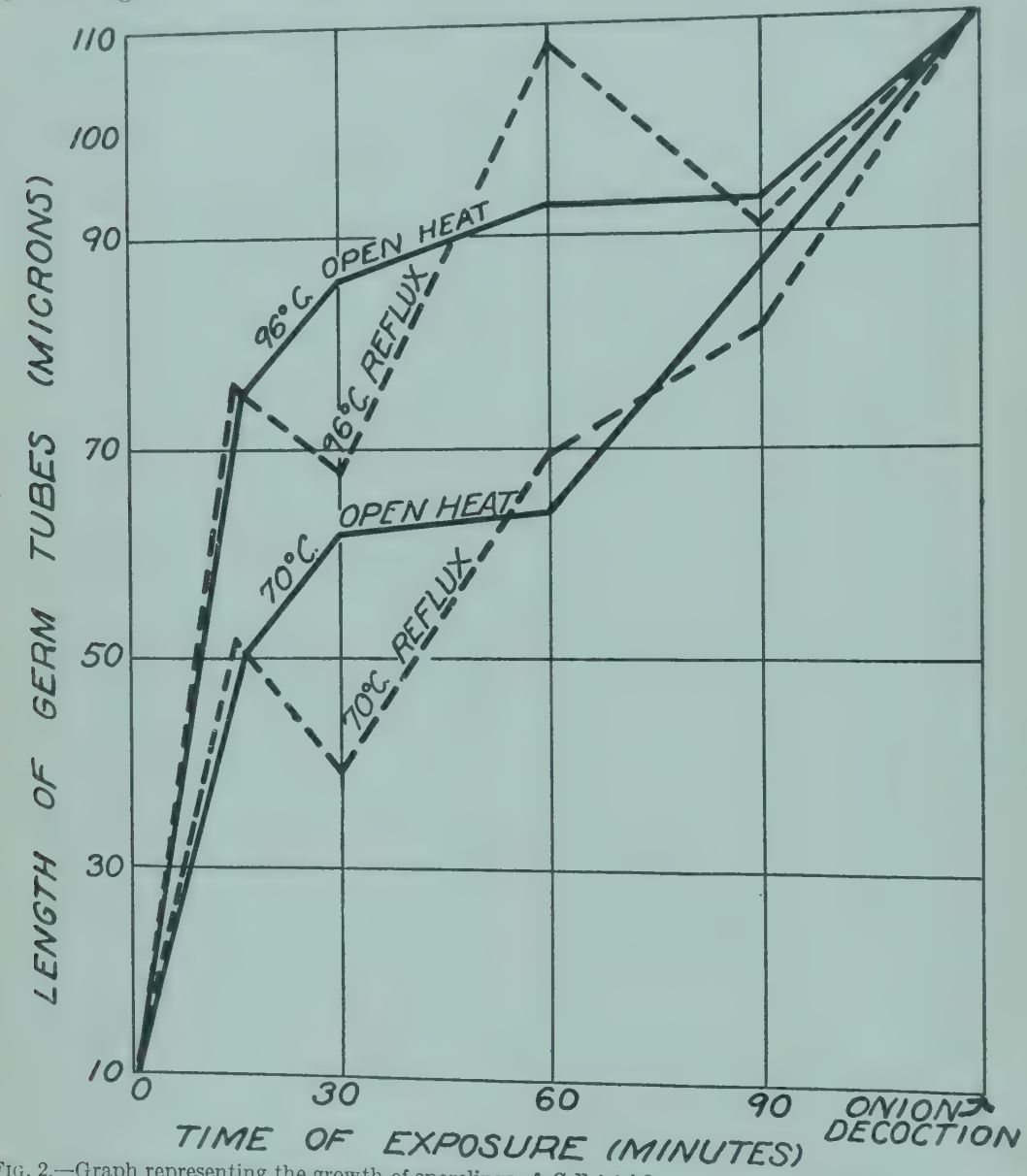

FIG. 2.—Graph representing the growth of sporelings of *Colletotrichum circinans* when exposed to two lots of onion juice which have been heated at 70° and 96° C., respectively, for various intervals either in an open vessel or in a vessel to which a reflux condenser had been attached. (See data in Table III)

germination of spores of *Colletotrichum circinans* so that no perceptible difference in the toxic action at various temperatures resulted. As a further safeguard, however, practically all of the experiments were run at a constant temperature of 25°.

result, under extremely moist conditions, and the cell lumen may be invaded before the cell collapses. Under ordinary storage conditions, however, the progress is very slow and at least in a majority of cases hyphae do not penetrate the living cells, but the latter

TABLE IV.—*Relation of temperature to the rate of depletion of the toxins in onion juice* [a]

Number of hours after extraction	Reaction of spores of *Colletotrichum circinans*									
	Juice kept in ice box					Juice kept at 25° C.				
	Reaction to volatile toxins				Spores placed directly in juice	Reaction to volatile toxins				Spores placed directly in juice
	2 drops	1 drop	0.1 drop	0.01 drop		2 drops	1 drop	0.1 drop	0.01 drop	
0	0	0	0	+	0	0	0	0	+	0
2					0	0	0	+	+	0
4					0	−	+	+	+	0
8					0	+	+	+	+	0
15	0	0	+	+	0	+	+	+	+	0
22	0	0	+	+	0	+	+	+	+	0
46	⊖	+	+	+	0	+	+	+	+	0
90					0					0

[a] Symbols used: 0=no germination; ⊖ =slight germination and retarded growth; −=medium germination and growth; +=good germination and growth.

ORGANISMS USED AND THEIR MODE OF ATTACK

For the extension of this study certain of the more common onion bulb parasites were considered. These, with the smudge fungus, were representatives of the four genera, Colletotrichum, Fusarium, Botrytis, and Aspergillus. To further supplement the comparative study one or more species from each of the first three genera and one from Glomerella, all of which were nonpathogenic to the onion, were added. As introductory to a consideration of further experimental data, the existing knowledge of the mode of attack of each of the onion parasites will be reviewed.

COLLETOTRICHUM

The onion smudge organism (*Colletotrichum circinans*) and its method of infection have already been described (*6*). Of importance in this connection is the fact that penetration always occurs directly through the cuticle by means of an infection hypha which is produced from an appressorium. Softening and invasion of the cell wall show signs of degeneration and collapse before invasion actually occurs. It appears, therefore, that this fungus, even after infection, seldom actually invades the living cells. These studies were made with northern Globe varieties. More recent observations of the White Bermuda variety indicate that it may be parasitized more readily by *C. circinans* than the northern varieties, but this is a matter still under investigation.

Other species of Colletotrichum and Glomerella were picked at random for comparison with *C. circinans*, none of them being parasitic upon onion.

BOTRYTIS

The onion neck-rot fungus (*Botrytis allii*) has been described by Munn (*3*). It is a wound parasite and ordinarily infects through injuries in the neck of the bulb. Munn has shown that this fungus secretes an enzyme which kills the bulb tissue in advance of the mycelium. Here also we have the organism living out of immediate contact with the living host cell.

A second species (*Botrytis sp.* 108a) [2] commonly occurs on the outer scales of white onion, causing what is known as

[2] For the purposes of this paper this form will be referred to as *Botrytis sp. 108a*. A full description of the species will shortly be published.

small sclerotial neck rot. As with *B. allii*, it usually enters through wounds at the neck but attacks the succulent scale tissues less rapidly than does *B. allii*.

Two strains of *Botrytis cinerea* Pers. were also included. One strain was isolated by the writers from cyclamen, the other was secured from G. B. Ramsey, who had isolated it from tomato. The parasitic behavior of this species has been described most recently by Brown (*1*). Its method of invasion on other plants is very similar to that described by Munn (*3*) for *B. allii*, except that *B. cinerea* may penetrate the leaf cuticle. Inoculation experiments with these strains on onion bulbs yielded only negative results.

FUSARIUM

Two strains of Fusarium (*Fusarium cepae* Han. and *Fusarium* sp. 45) isolated from onion and determined by inoculation to be capable of causing the common Fusarium bulb rot were used. For comparison, the wheat-scab organism, *Fusarium graminearum* Schwabe (*Gibberella saubinetii* (Mont.) Sacc.) was included. The onion Fusaria are parenchyma invaders. They enter principally through wounds at the base of the bulb and cause a semiwatery decay (*8*). The exact relation of parasite to host tissue has not been studied.

ASPERGILLUS

Two forms of Aspergillus were used. The black mold fungus (*Aspergillus niger* Van Tiegh.) is a common storage fungus, especially upon the southern Bermuda crop (*5*). The organism develops on the dead outer scales or between the succulent scales of the bulb, and the invasion of sound healthy scales is comparatively meager. Upon injection into wounds on the bulb it does not cause decay. It appears to have no marked preference between colored and white varieties of the Bermuda types.

A second species of Aspergillus, isolated from Italian garlic by E. D. Eddy, was used for comparison. This produces yellow spores and dark-brown sclerotia in abundance. Charles Thom of the Bureau of Chemistry, United States Department of Agriculture, to whom the culture was referred, is at present working upon the classification of this and related forms, and for the purposes of this discussion it will be referred to by his number, 4660. When spores and mycelium were injected into wounds in onion bulbs, a rapid decay resulted. In contrast to *A. niger*, then, it appears to be an active parasite of the onion bulb.

GERMINATION AND GROWTH IN ONION JUICE

It has already been shown (*7*) that ordinarily spores and mycelium of *C. circinans* neither grow nor survive in the undiluted juice expressed from onion scales. This is probably due for the most part to the residual, nonvolatile toxin which remains in the extracted cell sap. It is obvious therefore that the fungus hyphae would not invade the lumen of the host cell without some change in the contents of the latter being produced in advance of them or at least not without being protected by the plasma membrane of the host. It appears that changes in the host cell do occur in advance of the onion smudge organism (*6*). It was of interest to determine whether the extracted juice was similarly toxic to other bulb parasites. Spores of each of the fungi mentioned were placed in drops of expressed onion juice which was diluted to various degrees with distilled water. The results are recorded in Table V.

It was found that, with one exception, none of the spores germinated in undiluted juice. Very little germination occurred in a dilution of 1 to 1. It was generally true of all these fungi that germination and growth increased with greater dilution. This points to the presence of toxins, rather than lack of nutrient as the principal inhibitory factor, inasmuch as dilution decreases the amount of available food. In the dilution of 1 to 10 it is noteworthy that all three species of Colletotrichum were still completely inhibited, while the species of Botrytis and Fusarium which are pathogenic to onions germinated and grew. In these two genera the nonpathogenic species tried were still almost completely inhibited. It is interesting also to compare the two species of Aspergillus. *A. sp. 4660*, which is a vigorous bulb-decaying organism, grows well in the 1 to 10 dilution, while *A. niger*, a superficial fungus, is entirely inhibited. The differences in the behavior of the various species becomes less marked in the higher dilutions.

It is significant that of the onion parasites, the organisms causing the most rapid decay (*Botrytis allii*, *Fusarium cepae*, *Fusarium* sp. 45, and *Aspergillus* sp. 4660) show germination and growth in the 1 to 10 or in lower dilutions, while the least aggressive parasites (*Colletotrichum circinans* and

TABLE V.—*The effect of concentration of expressed onion juice upon germination and growth of various fungi*

Organism	Expressed onion juice					Onion decoction
	Undiluted	Diluted 1 to 1	Diluted 1 to 10	Diluted 1 to 100	Diluted 1 to 1,000	
Colletotrichum circinans:						
Percentage germination	0	0	0	91	89	84
Average length germ tube (microns)	0	0	0	123	164	165
Colletotrichum lindemuthianum:						
Percentage germination	0	0	0	0	1	57
Average length germ tube (microns)	0	0	0	0	--------	25
Colletotrichum pisi:						
Percentage germination	0	0	0	60	88	100
Average length germ tube (microns)	0	0	0	11	15	66
Glomerella cingulata:						
Percentage germination	0	28	50	26	56	75
Average length germ tube (microns)	0	1	1	1	2	37
Botrytis allii:						
Percentage germination	5	99	100	100	100	100
Average length germ tube (microns)	62	85	200	200+	500+	500+
Botrytis 108a:						
Percentage germination	0	99	100	100	100	100
Average length germ tube (microns)	0	300+	500+	500+	500+	500+
Botrytis cinerea:						
Percentage germination	0	0	80	100	100	100
Average length germ tube (microns)	0	0	9	244	500+	500+
Fusarium No. 45 (onion):						
Percentage germination	0	6	14	62	89	88
Average length germ tube (microns)	0	10	8	14	29	22
Fusarium cepae:						
Percentage germination	0	0	1	99	98	94
Average length germ tube (microns)	0	0	22	54	89	34
Fusarium graminearum:						
Percentage germination	0	0	1	95	96	100
Average length germ tube (microns)	0	0	12	33	75	137
Aspergillus 4660:						
Percentage germination	0	0	93	--------	--------	100
Average lenght germ tube (microns)	0	0	26	--------	--------	87
Aspergillus niger:						
Percentage germination	0	0	0	62	43	85
Average length germ tube (microns)	0	0	0	24	33	145

Aspergillus niger) show little or no germination and growth below the 1 to 100 dilution. In correlation with this fact it is found that the last two fungi neither occur as natural wound parasites nor do they cause infection when artificially inoculated into wounds. The other four organisms are normally wound parasites. As already noted by Walker and Tims (*8*), inoculation of onion bulbs with *Fusarium cepae* by injecting spores into fresh needle wounds usually yields negative results, unless the bulbs are kept in moist chambers for about 48 hours after inoculation. The exact nature of this phenomenon needs further study. It is probable that the cell sap exuding into a fresh wound has a great deal to do with the inhibition of this organism, which becomes a vigorous parasite when once established in the host tissue. Similarly the writers have noted in inoculation experiments with *Botrytis allii* that when onion bulbs, the necks of which have been clipped so as to expose freshly exuding cell sap, are sprayed with a spore suspension, sometimes an unusually small percentage of bulbs becomes diseased. When these organisms become established as in nature, either in somewhat more dilute cell sap or in meteoric water on the surface of the dead tissue at the neck of the bulb, it is evident that they overcome the toxic effects sufficiently to make progress.

EFFECT OF VOLATILE SUBSTANCES UPON GERMINATION AND GROWTH

Preliminary experiments on the effect of volatile onion oil upon germination and growth showed that all species were somewhat retarded. Following the methods already outlined and providing as uniform conditions as possible, the fungi were compared by using spores from fresh cultures, and exposing them to various amounts of toxin from a common source at a temperature of 25° C. Although many repetitions of this experiment were made, one representative set of data is presented for consideration. The results on spore germination are given in Table VI and those on growth of the sporelings in Table VII.

In comparing first the reaction of the several onion parasites (fig. 3), it is to be noted that there is again a rough negative correlation between aggressiveness of parasitism and sensitiveness to the volatile toxins. As evidenced both in germination and growth, *Colletotrichum circinans* is the most sensitive; *Aspergillus niger* is next; both of these are very weak parasites.

The reactions of the nonparasitic species do not lend themselves to any very general statement. Lack of pathogenic properties upon the onion is not necessarily correlated with high sensitiveness to volatile oils. In this case at least *Botrytis cinerea* was stimulated in germination and growth by exposure to the volatile oil. *Fusarium graminearum*, *Colletotrichum lindemuthia-*

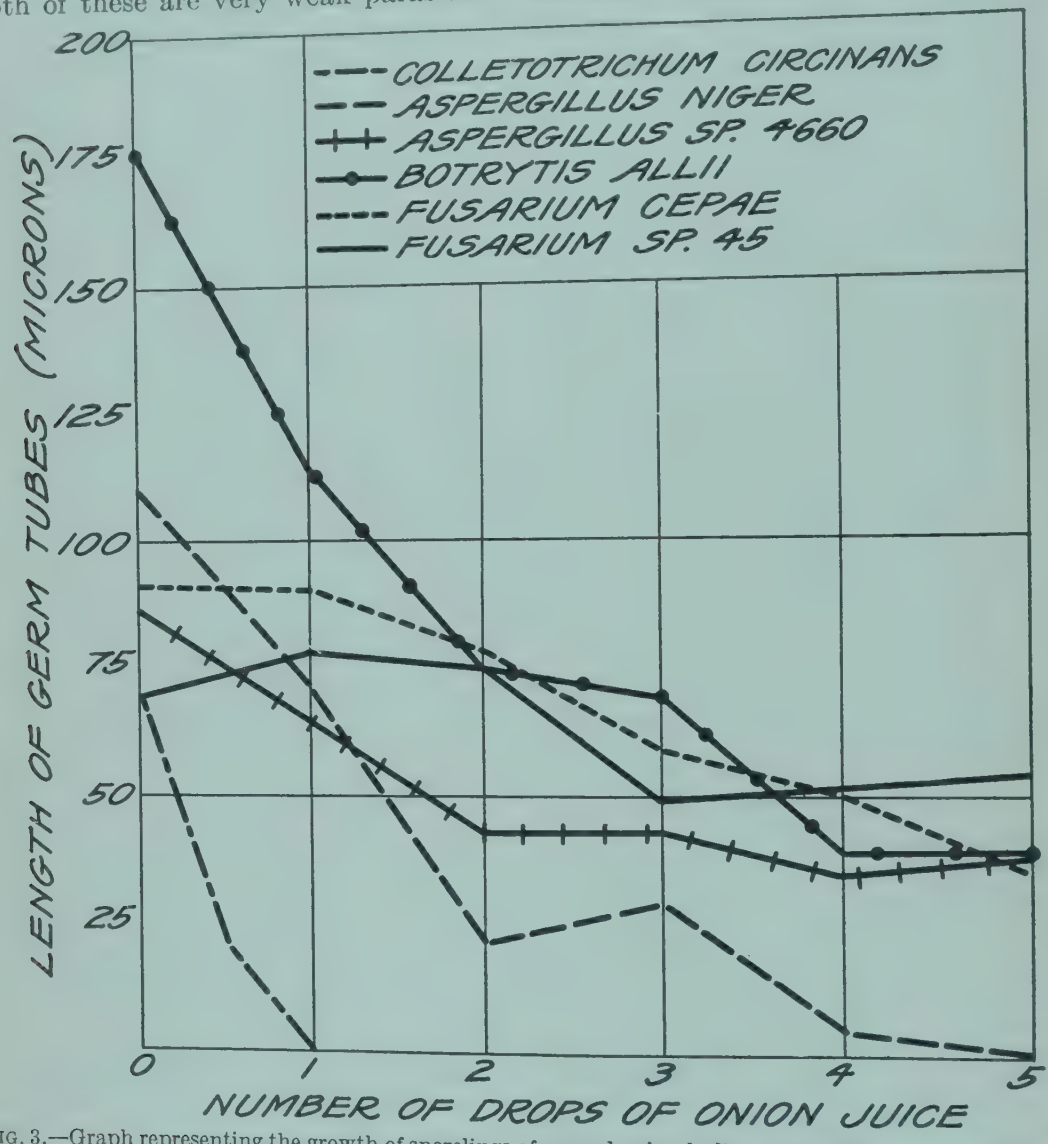

FIG. 3.—Graph representing the growth of sporelings of several onion-bulb parasites, when exposed to various concentrations of volatile onion oil. See data in Table VII. Note that the two weakest parasites, *Colletotrichum circinans* and *Aspergillus niger* are the most retarded by the volatile toxins as compared with the other four forms which are aggressive parasites of the onion bulb

In contrast to this, the more aggressive bulb-rotters (*Botrytis allii*, *Fusarium cepae*, *Fusarium* sp. 45, and *Aspergillus* sp. 4660) all show more or less germination and growth in the highest concentration of toxin. The trend therefore coincides in a general way with that noted for dissolved toxins (Table VI). Botrytis 108a occupies an intermediate position between the two groups, being a rather weak parasite, but still exhibiting considerable resistance to the volatile toxins.

num, and *C. trifolii* were distinctly inhibited, while *C. pisi* and *Glomerella cingulata* were only slightly retarded.

DISCUSSION OF RESULTS

In the establishment of the parasitic relation the factor most important to the invader is the presence of food, and one of the principal opposing factors is the presence of toxic substances within the host tissues. Since different species of fungi vary in their response to these antithetic factors, the question

TABLE VI.—*The effect of the volatile components of onion extract on the spore germination of several fungi*

Organism	Percentage of germination									
	Number of drops of onion juice in chamber									Control
	5	4	3	2	1	0.5	0.1	0.01	0.001	
Colletotrichum circinans	0	0	0	0	0	65	68	41	74	86
Colletotrichum lindemuthianum	0	0	0	0	0	0	77	60	64	57
Colletotrichum trifolii	0	0	0	0	40	17	30	27	------	38
Colletotrichum pisi	0	90	89	97	100	100	100	100	100	100
Glomerella cingulata	0	0	87	87	76	75	72	73	61	74
Botrytis allii	78	71	98	96	86	92	91	86	84	97
Botrytis 108a	90	------	90	95	100	100	100	100	100	100
Botrytis cinerea	71	78	83	63	37	23	9	29	24	30
Fusarium cepae	95	95	------	98	93	92	96	93	94	98
Fusarium No. 45 (onion)	30	------	64	86	70	------	94	94	96	89
Fusarium graminearum	0	0	0	0	100	95	90	97	96	98
Aspergillus niger	0	9	80	68	90	95	93	94	83	93
Aspergillus 4660	48	99	99	100	100	100	100	100	------	100

is pertinent to the relative importance of the latter in determining the degree of parasitism. There are several parasites of the onion bulb which vary in the mode and the aggressiveness of their attack. *Colletotrichum circinans* penetrates the cuticle readily at any point but once having gained entrance progresses very slowly, although it is not devoid of cellulose-digesting properties. *Botrytis allii* does not readily enter the unbroken surface but almost always enters through wounds or through dead neck tissues. Once within the host it readily kills the tissue slightly in advance of the hyphae and it is a much more aggressive parasite than *Colletotrichum circinans*. *Fusarium cepae* and *Fusarium* sp. 45 also gain entrance principally through wounds but cause very rapid decay having once entered. Two species of Aspergillus offer a similar contrast. One (*A. niger*) is very superficial and only mildly parasitic, while the other (*Aspergillus* sp. 4660) decays the tissue very rapidly.

These differences may originate in food relations, enzyme production by the invader, or antagonistic substances within the host. The present study is limited to the last question and has sought to determine what differential reactions on the part of bulb-rotting fungi and their allies occur in relation to toxic substances in onion tissue.

Inquiry into the nature of the inhibitory substances in the fleshy scales of the onion bulb shows that they are of at least two kinds. One type present in extracted juice is not readily removed or broken down by boiling and

TABLE VII.—*The effect of the volatile components of onion extract on the growth of sporelings of several fungi*

Organism	Length of germ tubes (microns)									
	Number of drops of onion juice in chamber									Control
	5	4	3	2	.1	0.5	0.1	0.01	0.001	
Colletotrichum circinans	0	0	0	0	0	22	28	25	43	72
Colletotrichum lindemuthianum	0	0	0	0	0	0	12	21	29	25
Colletotrichum trifolii	0	0	0	0	.6	9	13	14	0	12
Colletotrichum pisi	0	10	11	14	51	66	84	85	83	88
Glomerella cingulata	0	0	4	8	11	25	27	36	31	32
Botrytis allii	39	39	69	74	115	149	180	171	191	180
Botrytis 108a	151	------	197	215	184	228	197	176	223	185
Botrytis cinerea	14	22	21	35	57	50	58	80	62	50
Fusarium cepae	18	25	------	39	45	48	48	45	40	46
Fusarium 45 (onion)	18	------	16	25	26	------	24	22	25	23
Fusarium graminearum	0	0	0	0	44	90	132	166	170	170
Aspergillus niger	0	5	29	22	71	73	85	135	112	111
Aspergillus 4660	38	35	43	43	64	64	80	80	0	87

remains active apparently until the juice is destroyed by bacterial fermentation. The other type is volatile, and at ordinary room temperatures largely disappears from extracted juice within a few hours. It is destroyed by distillation at ordinary pressure. A third type of substance toxic to fungi is found in the dry outer scales of colored onion bulbs, and studies on it are published in other papers (*6*, *9*). While some of this third type may exist in the extract of fleshy colored scales it is probably too dilute there to be effective, and is considered distinct from the two types discussed here. These last two types appear to exist to about the same extent in white and colored onion bulbs.

By testing the reaction of these fungi of the two types of toxic substances under as nearly uniform conditions as possible some interesting results have been noted. The response to the two kinds of toxin is in general very similar for each fungus. Comparing onion pathogens and nonpathogens no strict negative correlation was found between pathogenicity upon onion and sensitiveness to the toxins. This simply indicates that other factors enter into the determination of the parasitic relation.

But to compare the onion pathogens as among themselves, it must be remembered that this method of exposure to the toxins is only an approximation to natural conditions. Whereas in our tests the fungus was exposed directly to the dissolved and volatile toxins, it is probable that in nature this exposure is not so severe nor direct. In the case of each of the bulb parasites considered here the host cells are affected somewhat in advance by the fungus enzymes and in this interaction the inhibitory effects of these host toxins are probably weakened. But in any case, it is plausible to suggest that these toxins may retard the advance of the parasite according to the specific sensitiveness of the latter. In support of this is the fact that *Colletotrichum circinans*, although it is able to penetrate the onion scales readily, is the most sensitive to the host toxins and is among the least aggressive as a parasite. *Aspergillus niger*, though slightly less sensitive to the toxins, is also decidedly limited in its properties as a decay-producing organism. *Botrytis allii*, once established, is one of the most aggressive of the bulb parasites, and in addition to its advantage of possessing more active cytoltic properties, it is also much less sensitive to the host toxins.

Botrytis sp. 108a is slightly less sensitive than *Botrytis allii*, although we should expect it to be somewhat more so should this factor be the only one which determines its weaker aggressiveness. In this case, and indeed in all the forms considered, further study should be directed toward a consideration of the enzym activities and food requirements of the organisms. The other active bulb invaders, *Fusarium cepae*, *Fusarium sp.* 45, and *Aspergillus sp.* 4660, fall into the general class with *Botrytis allii* as to their sensitiveness to the toxins and this is in correlation with their parasitic activity. It would appear from the evidence at hand that the toxic constituents of the host cell sap of the onion may exert an important influence on the aggressiveness of the fungi pathogenic to that plant. Any broader generalization at this time would not be justified.

SUMMARY

1. This investigation is a continuation of one previously reported in which was demonstrated the toxic nature of juice extracted from fleshy onion bulb scales toward the onion smudge organism (*Colletotrichum circinans*).

2. These toxins are of two general types, one which is neither removed nor broken down readily by heat and one which is volatile and passes off from the extracted juice at room temperature within a few hours.

3. There is a gradual decline during storage of onion bulbs in the amount of volatile toxin which may be released from the extracted juice. This decline is also evident on sprouting.

4. In general fungus spores become more sensitive with age to the volatile toxins.

5. The temperature at which spores are tested may have some influence on their reaction to the volatile toxins and it thus becomes essential to provide as uniform conditions as possible for comparative studies. The depletion of volatile toxins in onion juice is hastened by increase in temperature.

6. The primary purpose of the investigation was to compare representative onion bulb parasites with one another and with other fungi nonpathogenic to onion in their reaction to these toxins. The following bulb parasites were included: *Colletotrichum circinans*, *Boytrytis allii*, *Botrytis* sp. 108a, *Fusarium cepae*, *Fusarium* sp. 45, *Aspergillus niger*, *Aspergillus* sp. 4660. Nonparasitic forms were: *Colletotrichum lindemuthianum*, *C. trifolii*, *C. pisi*, *Glomerella cingulata*, *Botrytis cinerea*, and *Fusarium graminearum* (*Gibberella saubinetii*).

7. In general, the reaction of each fungus followed the same trend for the dissolved as for the volatile toxins.

8. Comparing onion pathogens and nonpathogens, there is no strict negative correlation between pathogenicity upon onion and sensitiveness to the toxins, which only indicates that other factors enter into the determination of the parasitic relation.

9. Considering the onion parasites as between themselves there is evident a negative correlation between aggressiveness of parasitic attack and sensitiveness to the dissolved and volatile toxins. It is pointed out that the experimental results are not truly representative of the actual relation between host and parasite. The presumption, however, is that as the parasite invades the tissue the host toxins, though attenuated by fungous enzymes, may possibly exert some retarding effects upon the invader. If this be the case, it is suggested that the host toxins may be one of the numerous factors which determine the degree of parasitism attained by a given parasite.

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BORAX AS A DISINFECTANT FOR CITRUS FRUIT[1]

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The use of disinfectants in washing citrus fruits has been a common practice in California for many years. In 1906 Smith[2] showed that the brown-rot of lemons caused by *Pythiacystis citrophthora* Smith and Smith, could be controlled by a solution of potassium permanganate, copper sulphate, or formalin in the wash water. Since then copper sulphate has been used to a considerable extent commercially. For fifteen or twenty years hot water and soap have been employed in washing both oranges and lemons, and for the past ten years a soap containing borax has been extensively used. It has also been a common practice in some packing houses to use a dilute solution of borax in the wash water in addition to the soap. Except in the case of brown-rot of lemons, there appears to be no data on the exact effect of these various disinfectants in the control of the fungous diseases of Citrus which gain entrance to the fruit in handling and harvesting operations and are particularly evident during transit and on the market.[3] To obtain more exact information upon the possibilities of controlling some of the common Citrus parasites such as blue mold, caused by *Penicillium italicum* Wehmer, and green mold, caused by *Penicillium digitatum* Sacc., a series of experiments was conducted in which some common and cheap disinfectants were used in the wash water. Since the results of these investigations were fairly definite and present a basis for further commercial application of disinfectants in the control of certain Citrus diseases, a preliminary presentation is here given.

The work was begun in California early in 1924, either Navel or Valencia oranges being used in all experiments described in this paper. In the first preliminary experiment, powdered boric acid U. S. P. and potassium alum Tech. were used. Navel oranges, which had been picked from four to six weeks, were prepared for the experiment by removing thin slices of skin from each fruit. Seventy-five fruits were immersed for five minutes in a solution of 2.5 per cent of boric acid at a temperature of 120° F.; two other lots of 75 each were treated, one by immersing in a solution of 4.5 per cent powdered alum and the other in hot water only for five minutes at 120° F. After the fruits had been removed from the baths they were inoculated with spores of blue mold obtained from a decayed fruit. The fruit was dried, packed, and stored in a room having a temperature of about 70° F. and a humidity of about 90 per cent. After one week both the fruit treated with hot water and that treated with powdered alum solution showed 100 per cent decay caused by blue mold. The lot treated with boric acid showed only 2.5 per cent decay from blue mold and about the same percentage from green mold. Further holding of the lot showed some decay from what seemed to be *Alternaria* sp. or *Pythiacystis* sp. In this connection it is interesting to note that Smith[4] found that very dilute solutions of boric acid would not control *Pythiacystis citrophtora* on lemons.

This experiment indicated that boric acid offered very promising possibilities and that alum was apparently of no particular value in concentrations such as could safely be used in washing the fruit. Borax (technical grade) was later tested and found to be as effective as boric acid in controlling blue-mold decay, and, being much cheaper, was used in all later experiments. The investigation here described was concerned mainly with testing the efficiency of a 2.5 per cent solution of borax at various temperatures and with different methods of application in the control of blue and green mold which had been inoculated

[1] Received for publication Aug. 31, 1924; issued April, 1925.

[2] SMITH, R. E., and others. THE BROWN-ROT OF THE LEMON. Calif. Agr. Exp. Sta. Bul. 190, 72 p., illus. 1907.

[3] POWELL, G. H., and others. THE DECAY OF ORANGES WHILE IN TRANSIT FROM CALIFORNIA. U. S. Dept. Agr., Bur. Plant Indus. Bul. 123, 79 p., illus. 1908.

[4] SMITH, R. E. OP. CIT.

on the surface of wounded fruit and in punctures similar to those made by stem or thorns.

The fruit was prepared for inoculation either by cutting thin slices of the rind just below the oil cells and into the white portion similar to the wound made by a clipper cut or by puncturing the rind with a knife to the depth of about 2 mm. The depth the knife was thrust into the fruit was controlled by wrapping tire tape around the blade so that it could penetrate the fruit only to the depth required. The inoculations were made by rubbing spores on the cut surface, where a portion of the rind was sliced away, and by dipping the knife in a mass of spores when making the wound by puncture. The spores for inoculating the fruit were obtained from decayed oranges. Since the greater part of this work was done under ordinary packing-house conditions and with the regular packing-house machinery, no attempt was made to get pure cultures of the fungi for making inoculations. Decayed oranges were selected, however, as the source of spores, because judging from the external character and color (*1*, *3*), these seemed to furnish practically pure cultures of the fungi.[5] In many cases, undoubtedly, the fruit was inoculated from mixed cultures and there was especially in the controls considerable possibility of natural inoculations when the regular packing-house machinery was used in handling the fruit.

In the early experiments the procedure was to wound the fruit, treat it with a 2.5 per cent solution of borax, either by immersing or floating it in this solution, inoculating it by rubbing a finger dipped in the blue mold spores on the cut surface, drying in the air, wrapping, packing, and storing. The fruit was usually stored at about 70° F. with a humidity of about 90 per cent. Controls were prepared in exactly the same way except that instead of being treated with the borax solution they were treated with water only and inoculated with the fungi. At the end of one week the fruit was examined, the decayed fruits classified and removed, and the remaining sound fruits allowed to remain for the next inspection. When the fruit was immersed, it was held about 4 inches below the surface of the solution by a rack. When it was floated no weight was used, and it was allowed to float freely in the tank of solution, from one-eighth to one-tenth of the rind being exposed above the surface. The results of the experiments made with blue mold fungus on Valencia oranges are shown in Table I.

Table I shows that there is much more decay in the untreated fruit after one week than in any of the borax-treated lots. In the latter the best results were obtained at the higher temperatures and when the fruit was allowed to remain for four minutes or more in the solution. In no case was there more than 4.5 per cent of blue mold in any of the lots treated at 120° F. except in the two lots sprayed with water after being treated with the borax solution. In these lots the decay caused by blue mold after one week was 6.1 and 7.8 per cent. Evidently for the best results the borax solution should be allowed to dry on the fruit. At the higher temperatures blue mold is apparently controlled as well by allowing the fruit to float in the borax solution as by immersing it. At the lower temperatures, however, floating did not give as good results as did immersion. In the lots of fruit injured by puncturing and inoculating in these wounds, practically as good control was obtained as when the wounds were superficial.

The results of the inspections after two weeks show some increase in the amount of blue mold present in the treated fruit, although in the lots treated at 120° for eight minutes there was in no case more than 5 per cent of blue mold decay. There was little increase in blue mold decay after five to seven weeks. The amount of decay caused by green mold was very much higher during the later inspections than at the first. This fungus is apparently not so easily controlled by borax as is the blue mold fungus. Results of a series of experiments on the treatment of Valencia oranges inoculated with green mold are shown in Table II. The spores for inoculating this fruit were obtained from decayed specimens which appeared to be affected only with green mold, although, of course, as in the case of blue mold there was probably some contamination.

An inspection of Table II corroborates the observation indicated by Table I, namely, that green mold is not readily controlled by treatment with this concentration of borax. In the lots of fruit wounded on the surface and immersed, there was no apparent infec-

[5] THOM, C. CULTURAL STUDIES OF SPECIES OF PENICILLIUM. U. S. Dept. Agr., Bur. Anim. Indus Bul. 118, 109 p., illus. 1910.

TABLE I.—*Showing percentage of decay in Valencia oranges, wounded on surface or punctured, immersed or floated in a 2.5 per cent solution of borax, inoculated on the wounds with blue-mold spores, and held in a warm room at high humidity*

Treatment	Temperature bath	Time	Number of fruit	Percentage of decay after— One week		Two weeks		Five weeks		Seven weeks	
				Blue mold	Green mold	Blue mold	Green mold	Blue mold	Green mold	Blue mold	Green mold
	°F.	*Minutes*									
Control wounded and inoculated but not treated with borax			73	98.6	0						
			100	100.0							
			513	90.0							
			686			92.4	0				
			100	100.0							
Wounded on surface, immersed in borax solution and inoculated	73	4	100	13.0	17.0	14.0	65.0	14.0	84.0		
	73	8	13	0	0	0	15.3				
	120	3	224	4.4	2.6						
	120	3	459			9.3	32.4				
	120	3	224	4.5	0						
	120	4	75	2.6	2.6						
	120	4	15	0	6.6						
	120	4	395	2.2	1.2						
	120	4	972			12.7	36.0				
	120	4	485	2.3	0						
Wounded on surface, floated in borax solution and inoculated	73	4	100	41.0	16.0	42.0	48.0	42.0	54.0		
	120	8	423	0	0						
	120	8	488			4.9	25.8				
Wounded on surface, immersed in borax solution, inoculated, and rinsed with water	73	4	50	26.0	16.0	26.0	38.0	26.0	60.0		
	73	6	50	60.0	8.0	64.0	24.0	64.0	36.0		
Wounded on surface, immersed in borax solution, inoculated and sprayed with water	120	6	587	6.1	6.8						
	120	6	701	7.8		45.1	30.1				
Control punctured and inoculated; not treated with borax			250	83.6	0	84.8	0	86.0	0		
Wounded by puncturing, inoculated and immersed in borax solution	70	8	50	0	0	2.0	0	2.0	4.0	2.0	4.0
	120	4	100	2.0	0	6.0	4.0	6.0	46.0	7.0	53.0
	120	6	100	0	0	2.0	3.0	2.0	34.0	3.0	40.0
	120	8	150	3.3	0.6	4.6	2.6	5.3	30.0	5.3	37.9

TABLE II.—*Showing percentage of decay in Valencia oranges, wounded on surface or punctured: immersed or floated in a 2.5 per cent solution of borax, inoculated with green mold spores, and held in a warm room at high humidity*

Treatment	Temperature bath	Time	Number of fruit	Percentage of decay after— One week		Two weeks		Five weeks		Seven weeks	
				Blue mold	Green mold	Blue mold	Green mold	Blue mold	Green mold	Blue mold	Green mold
	°F.	*Minutes*									
Control wounded on surface and inoculated, not treated with borax			100	80.0	20.0						
Wounded on surface and immersed in borax solution	67	4	100	0	27.0	0	97.0	0	99.0		
	67	8	100	0	15.0	0	92.0	0	95.0		
	120	4	100	0	24.0	0	92.0	0	98.0		
	120	8	100	0	2.0	0	50.0	0	73.0	0	80.0
Control wounded by puncturing, inoculated, not treated with borax			37	0	100.0						
Wounded by puncturing, inoculated and immersed in borax solution	73	4	100	11.0	2.0	15.0	2.0	18.0	24.0	19.0	28.0
	73	6	100	16.0	0	18.0	7.0	21.0	45.0	22.0	50.0
	70	8	46	0	8.7	0	48.0	0	84.8		
Same except treated by floating in borax solution	73	4	50	8.0	0	8.0	4.0	10.0	42.0	10.0	44.0
	73	6	50	14.0	0	18.0	4.0	18.0	26.0	18.0	32.0

tion from blue mold but at the end of five weeks 73 per cent or more of the fruit had decayed from what apparently was green mold. With the fruit inoculated by puncturing and immersion, the results are not so striking; there is considerably more blue mold present, indicating contamination of the culture used for inoculation. The percentage of rot caused by green mold as shown by the later inspections is high in all cases, although it was above 50 per cent in only one case.

From the results of the experiments herein reported it is evident that blue mold rot, which causes so much damage to Citrus fruit in California, can be largely controlled by treating the fruit with a solution of borax. Much of the work described in this paper was done in the packing house and with equipment suitable for application in a commercial way. It remains, however, to make further experiments on a commercial basis, to determine the value of the treatment in actual shipping and marketing tests. While borax [6] in the concentration used is not so effective in the control of green mold as in that of blue mold, it is probable that higher concentrations of borax or other disinfectants may be found which will be effectious against this fungus. Work along these lines is in progress.

[6] The question as to whether treatment with borax should be indicated by special label or otherwise for nterstate shipment has not yet been determined.

THE OCCURRENCE OF COPPER, MANGANESE, ZINC, NICKEL, AND COBALT IN SOILS, PLANTS, AND ANIMALS, AND THEIR POSSIBLE FUNCTION AS VITAL FACTORS[1]

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INTRODUCTION

A problem of fundamental importance in agricultural research to-day is to ascertain which of the elements present in small quantities in soils, plants, and animals are necessary in the vital processes and what are their functions. The purpose of this paper is to show that some of the so-called nonessential elements are of common occurrence in Kentucky soils and in certain tissues of plants and animals produced on such soils and that their concentration is greatest in certain vital organs of plants and animals, a coincidence which suggests the possibility that such elements may have important functions in the life processes.

HISTORICAL DATA

For a long time it has been taught and accepted that available compounds of only 10 elements are all that are necessary for the normal growth and maturation of agricultural crops. It is a well-known fact, however, that a much larger number of elements than 10 occur in small amounts in fertile soils and in the ashes of normal plants that have grown in the soil. Palladin (*13*, p. 82)[2] names 31 elements that have been found in the ashes of plants grown under natural conditions in the soil. The 10 elements which have heretofore been considered sufficient for the growth of plants are carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, and iron.

Within the past 50 years many investigations have been recorded in chemical literature which show that the elements arsenic, antimony, cadmium, copper, manganese, zinc, nickel, cobalt, barium, strontium, bromine, and iodine are widely distributed in soils and plants. In recent experiments observations have been made which indicate that a few of the so-called nonessential elements may have more important functions in soils, plants, and animals than is generally recognized.

Greaves (*3*, p. 119) states that arsenic is a constituent of virgin soils and that certain compounds of this element stimulate the processes of ammonification, nitrification, and nitrogen fixation in the soil.

In 1866 Neubauer (*12*) published a method for the estimation of copper in the tissues of plants and animals. Later investigations show that this element is widely distributed in natural waters, soils, plants, and animals. Certain species of mollusks are known to be relatively rich in copper.

Willard (*16*) reports that in 34 different samples of oysters collected at different points along the East Coast of the United States a minimum of 50 parts per million and a maximum of 1,700 parts per million of copper was found in the moisture-free matter.

Bertrand (*2*) has published results which show quite appreciable amounts of arsenic, boron, iodine, copper, manganese, zinc, and fluorine to be of common occurrence in the tissues of plants and animals. He also offers the suggestion that copper, manganese, and vanadium may replace the function of iron in certain mollusks, crustacea, and tunicates.

Benzon (*1*) has recently published data from which he concludes that zinc functions as the so-called water-soluble vitamin B.

Javillier (*5*, *6*, *7*) has shown that zinc is a constituent of normal plants and that conifers are relatively rich in this element. He concludes that plants which contain chlorophyll are benefited by the action of small amounts of zinc which perhaps act as a catalytic agent in the metabolic processes.

Van Itallie and Van Eck (*4*) state that copper and zinc appear to be regular constituents of the liver. These metals were found in the newly born.

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[2] Reference is made by number (italic) to "Literature cited," p. 196.

In a stillborn child there were 26.1 mg. of copper and 73.9 mg. of zinc per kilogram in the liver.

The author (*8, 9, 10, 11*) has shown that manganese is an essential element in the growth of autotrophic plants and functions in the synthesis of chlorophyll, for when this element is carefully excluded from sand culture experiments plants do not synthesize chlorophyll and, consequently, make very little growth.

EXPERIMENTAL DATA

SOILS

Preliminary experiments on a few virgin soils from the more important soil areas of Kentucky show that they contain amounts of the elements arsenic, copper, antimony, manganese, zinc, nickel, cobalt, barium, and strontium large enough for estimation by the usual methods of qualitative chemical analysis applied to the hydrochloric acid extract from 1,000 gm. of air-dried soil. The purified precipitates obtained in the analysis of the solution from one virgin soil gave the following quantitative results in parts per million of the element in the air-dry soil:[3] Arsenic 10.8, copper 7.2, zinc 27.7, cobalt 1.5, nickel 3.9. The presence of these elements in a Kentucky soil in greater quantities than was anticipated suggested further investigations as to their presence in certain parts of plants and animals which had received their sustenance from the soil in which these elements occur.

PLANT SUBSTANCES

Thus far the foliage and seeds of a few of the more important farm crops have been examined for some of the so-called nonessential elements. The ash resulting from the incineration of 1,500 gm. of air-dry corn silage was digested in hot 20 per cent hydrochloric acid. The insoluble material was filtered and washed free of chlorides. The hydrochloric acid solution of the ash was examined by the usual procedure of qualitative chemical analyses and the following elements were found: Arsenic, appreciable; lead, trace; copper, appreciable; manganese, considerable; zinc, appreciable; nickel, trace; barium and strontium, appreciable.

Samples of the following materials were analyzed:

Kentucky bluegrass (*Poa pratensis*): A clean, hand-picked sample of the fresh blades which were about 6 to 10 inches in length. The blades of grass were free from soil or other extraneous matter.

Soybean leaves: Fresh leaves were hand picked from the upper half of plants about 3 to 4 feet tall, growing in the field. The leaves were free from soil or other visible extraneous matter.

Soybean seeds: The seeds were clean and mature, and were grown on the same farm and the same type of soil as the soybean leaves but not on the same plants.

Wheat bran: A sample of wheat bran from a red winter wheat was obtained at a flour mill. The bran was freed from the germs and small particles of bran by shaking in a 20-mesh sieve and selecting the larger flakes of bran that remained on the sieve for the analysis.

Wheat germs: This material was not absolutely pure wheat germs but was a representative sample of the material designated as wheat germs by the miller. It contained small particles of bran and some flour. Perhaps the germs formed as much as 70 per cent of the mixture.

Patent flour: The sample represented the highest grade of patent flour and was produced at the same mill at which the wheat bran and wheat germs were obtained.

Corn germs: The corn germs were dissected by hand from sound, mature grains of white Hickory King corn, which had grown on the Kentucky Agricultural Experiment Station farm, at Lexington.

Corn endosperm: The remaining part of the corn grains was used from which the germs had been cut out by hand with a small gouge.

Rice polishings: A sample of the commercial material containing the pericarp and germ of the rice grain, removed from the grain in the process of polishing.

Polished white rice: The commercial product from which the pericarp and germ have been removed in the process of polishing was selected.

It is evident from the data in Table I that when the cereals, wheat, corn, and rice are highly milled by modern processes, the finished products, patent flour, degermed corn meal, and polished rice, are practically freed from copper, manganese, zinc, and iron. It is interesting to note that the germs of wheat and corn are relatively rich in copper, manganese, and zinc, thus showing a close association of these elements with the vital part of these grains.

[3] Methods of estimation (*14*): Arsenic, Gutzeit Method, page 46; Copper, ammonia method, page 167; Zinc, precipitated as sulphide in CH_3COOH and weighed as sulphate, p. 479; Nickel, dimethylglyoxime method, p. 287; Cobalt, nitroso-beta-naphthol method, p. 143

TABLE I.—*Metals found in vegetable substances, expressed in parts per million of moisture-free material*[a]

Material	Copper	Iron	Manganese (*15*)	Zinc	Nickel	Cobalt
Bluegrass (*Poa pratensis*)	7.5	336.0	30.0	28.0	Trace	Trace
Soybean leaves	8.0	336.0	160.0	110.0	Trace	Trace
Soybean seeds	12.0	70.0	32.5	18.4	3.92	Trace
Wheat bran	16.0	210.0	125.0	75.0	----------	----------
Wheat germs	46.0	270.0	150.0	160.0	----------	----------
Patent flour	Trace	24.0	10.0	Trace	----------	----------
Corn germs	10.0	270.0	40.0	103.0	----------	----------
Corn endosperm	Trace	30.0	16.0	Trace	----------	----------
Rice polishings	7.0	168.0	100.0	70.0	----------	----------
Polished rice	Trace	3.0	10.0	Trace	----------	----------

[a] The data in Table I, except those for manganese, were obtained by methods described by Scott (*14*), using the hydrochloric acid solution of the ash from 100 gm. of air-dried material, except the soybean seeds, of which 500 gm. were used. Manganese was determined by the periodate method (*15*) in a separate portion of 10 gm. of material.

TABLE II.—*Metals found in animal substances; expressed in parts per million of the moisture-free material*

Material	Copper	Iron	Manganese	Zinc
Lean meat of ox	0.4	225.0	Trace	15.0
Liver of same ox	75.0	308.0	15.0	112.0
Liver of calf one week old	345.0	168.0	14.0	122.5
Blood of same calf	8.0	1,720.0	Trace	32.0
Testicles of a mature horse	154.0	221.0	12.0	55.0
Egg yolks	2.5	100.0	1.5	67.0
Cod liver chum	44.0	173.0	4.0	92.0

ANIMAL SUBSTANCES

The foregoing observations and results on the occurrence of copper, manganese, and zinc in the foliage and seeds of important agricultural plants suggested further investigations on their occurrence in the tissues and fluids of domestic animals. The method of procedure used for all animal substances was substantially the same as the following, used for lean meat of an ox. A sample of lean meat from a freshly slaughtered mature normal ox was obtained, run through a meat chopper, and dried at 100° C. One hundred grams of the moisture-free lean meat was heated gently in a clean quartz dish until no further volatile matter was given off. The carbonized matter was then digested with hot 20 per cent hydrochloric acid and ground fine in a porcelain mortar, transferred to a Buchner funnel, filtered and washed with hot water. The remaining residue of carbonized matter was returned to a quartz dish and further ashed at a temperature below redness in a muffle furnace. This method of alternate ashing and extraction with acid and water was repeated until all the carbon was consumed and the mineral matter contained in the meat was in solution. The solution was made to a definite volume and aliquots taken for the estimation of the different elements reported in Table II

DISCUSSION OF RESULTS

From the results contained in this preliminary report it is evident that small amounts of copper, manganese, zinc, nickel, and cobalt are widely distributed in soils and plants. Each of these elements has been found in larger amounts than was anticipated in samples of soil from a few of the larger geological formations in Kentucky.

Examination of plants grown under natural conditions in the soil have revealed the presence of copper, manganese, and zinc in every case and in larger amounts than were anticipated. Nickel and cobalt occur in some of these plants in sufficient amounts to permit quantitative determination of these elements when not more than a kilogram of the dry plant material is ashed and these elements are carefully sought, using standard methods of chemical analysis.

The fact that the liver of a young calf contained so much copper is both interesting and significant. Since the calf was only a week old it must have obtained all this copper from the mother cow. The calf was apparently normal in every respect at the time it was slaughtered. The fact that the calf absorbed the amount of copper that it contained from the mother cow raises the question as to the effect on the offspring if the food of the mother cow

had been deficient in either copper, manganese, or zinc. The author is seeking a definite answer to this question by experiments both with plants and with small animals. It has been known for a long time that copper is of common occurrence in the tissues of land and marine animals. It is stated that copper replaces the function of iron in the metabolism of some of the lower forms of animal life. It is a well known fact that inorganic compounds of copper, manganese, and zinc have important catalytic functions and it is not unreasonable to assume such a rôle for the organic colloidal complexes of these metals in the metabolic processes of plants and animals.

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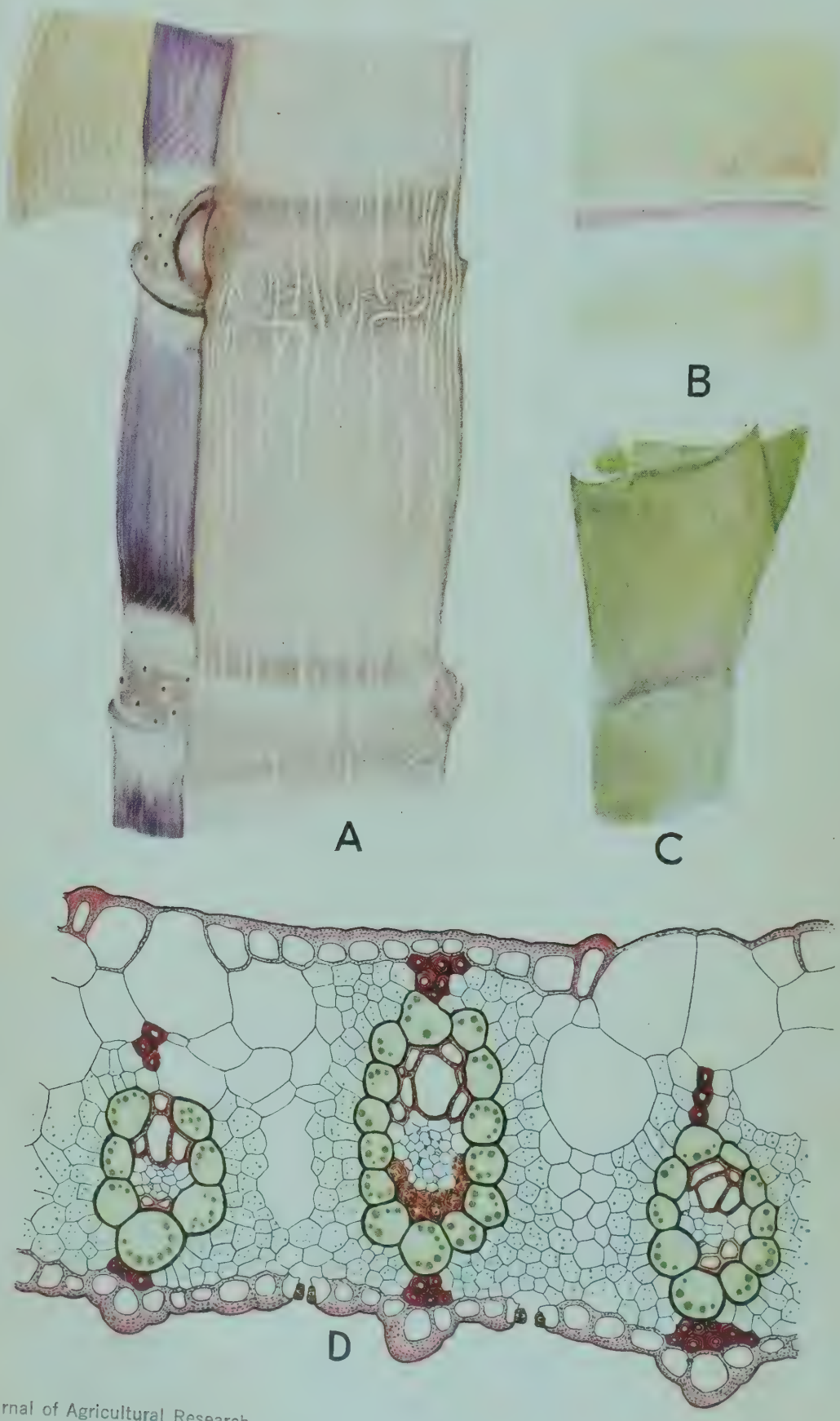
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ANATOMY OF THE VEGETATIVE ORGANS OF SUGAR CANE [1]

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INTRODUCTION

The vegetative organs of the grasses have been the subject of very extensive studies, and certain structures, like the vascular bundles of the stem, are familiar to elementary students of botany. The more difficult features of the inner structure of this group of plants have also received the close attention of plant anatomists, but it was only after many unsatisfactory attempts that a correct understanding was reached, as, for example, in the account by von Mohl (*13*)[2] of the relationship of the leaftraces. The peculiar and variable structure of the leaves was made the object of extensive studies especially by Duval-Jouve (*5*). Strasburger (*16*) has given an excellent account of the course of the leaf traces in *Zea mays*, a plant which in many respects is closely akin in structure to the sugar cane. The works of Kobus (*10*), Beneke (*1*), Wiesner (*19*), Krüger (*11*), Geerts (*6*), Dickhoff (*4*), Kuyper (*12*), Bremekamp (*2, 3*), Kamerling (*8*), Venkatraman (*18*), and Takenuchi (*17*) deal with certain phases of the anatomy of sugar cane (*Saccharum officinarum* L.), and fragmentary accounts are found here and there in connection with studies of sugar-cane diseases.

Additional work on the anatomy of sugar cane has seemed desirable, since none of the existing publications give a complete and sufficiently detailed picture of the inner structure of the plant, and even these publications are accessible to but few.

THE PLANT

The variety Louisiana Purple (Black Cheribon) (pl. 1) which has been selected for study is a representative of the large group of varieties within the species *Saccharum officinarum*, or the so-called "noble canes" as distinguished from the more slender-stalked prolific-stooling groups of varieties, of which the well-known Uba is typical, and from the well-defined group of exceedingly thin-stemmed canes of northern India, like the Chunnee and Ruckee. The two latter groups of varieties exhibit differences which probably entitle them to specific rank, according to J. Jeswiet of the Proefstation voor de Java-Suikerindustrie.[3] The Louisiana Purple variety is a stout grass with solid juicy stems, broad flat leaves and large plumelike inflorescences.

The stem is from 3 to 5 m. tall and about 4 cm. thick; it is composed of nodes and internodes. The latter are commonly barrel-shaped or cylindrical and disposed linearly one above the other. They are of even thickness except in the underground part, where they taper to a cone (fig. 1).

[1] Received for publication May 2, 1924; issued April, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 221.

[3] Not published but communicated verbally by Jeswiet to E. W. Brandes, pathologist in charge, Office of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Dept. of Agriculture.

EXPLANATORY LEGEND FOR PLATE 1

Anatomy of the Vegetative Organs of Sugar Cane.

A.—Node and internode of mature stem of Louisiana Purple. The colors are natural except in the cut surface; the latter is a reproduction of the colors obtained by leaving the material in potassium bi-chromate. The vascular bundles appear white.

B.—Base of leafsheath. The sheath-joint appears as a light colored and smooth, somewhat protruding shoulder.

C.—Blade joint; the flanges of the joint are brown and pubescent.

D.—Cross section through a mature leaf. The chloroplasts are colored green; the lignified tissue red. ×234.

The internodes tend to increase in length acropetally, but in the apical region they become shorter again. The nodes are commonly thinner than the internodes. Above them, protected by the sheath, is the Keimring, which bears a bud and several rows of root primordia. All parts of the stem, with the exception of the Keimring, are covered with wax. In old stems these wax deposits peel off in places, thereby often giving the surface of the cane a peculiar striation.

The buds in this variety appear as appressed conchate structures of varying form. They are slightly sunken in the stem, as indicated by a longitudinal groove, which only gradually loses itself in the upper part of the internode. In the lower, more mature parts of the stem the buds become more prominent, and the tips bend away from the axis instead of lying next to it.

The leaves are two-ranked and parallel-veined. Each leaf consists of two parts—the sheath and the blade. The sheath envelopes the stem with the margins overlapping, and as a result of this arrangement one leaf will form a right, the next in succession a left spiral. The sheath is widest at the base and gradually narrows, reaching its smallest diameter where it merges into the blade. The base of the sheath is swollen, forming a pseudojoint. At the junction of sheath and blade a similar joint is formed. On the inside of this blade joint there is a membranous, hyaline appendage, the ligule. The sheath has an average length of about 34 cm. and a width which is directly correlated with the thickness of the stem. In a given region the sheath is thickest at the middle, becoming gradually thinner toward the margins. The surface of the sheath is covered with hairs of

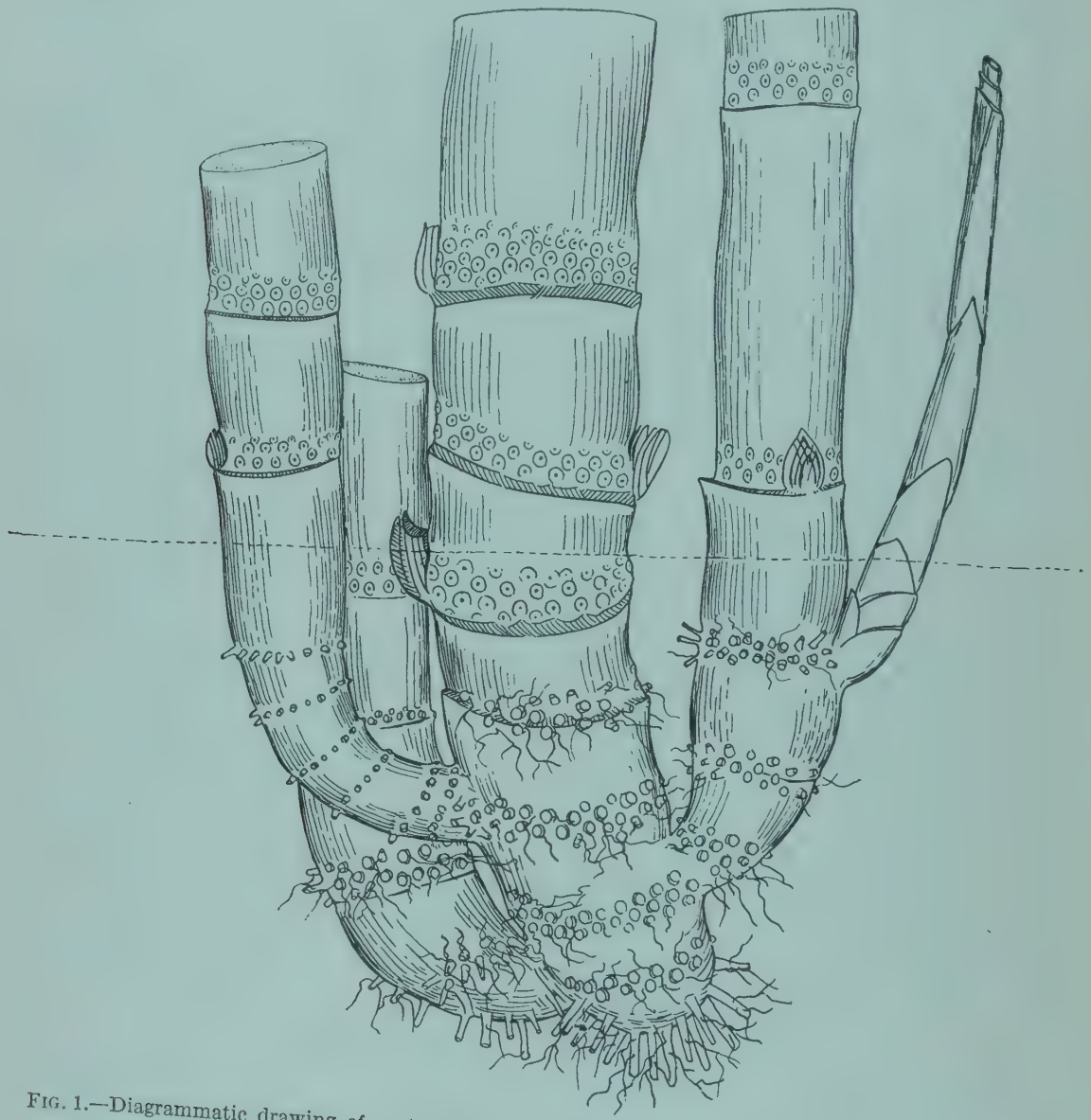


FIG. 1.—Diagrammatic drawing of part of stool of large sugar cane. New secondary and tertiary stems arise from the buds of the underground stem

various types. These are most numerous at the two joints and along the median axis. At the base of the ligule, but arising from the inner epidermis of the sheath, are long silky cilia.

The blade is linear lanceolate, up to two meters long, and at the middle is from 5 to 7 cm. wide. There is a prominent midrib which projects from the lower surface and forms a groove in the upper. The leaf margin is denticulate with fine silicified teeth. The surface varies from hispid to pubescent with the longer, softer types of hair occurring on the lower surface.

The color of the cane is characteristic of the variety. Gradations, however, are induced by light and the age of the organ. The sheath is of a uniform green, which becomes pale toward the margins. Sometimes the basal part of the young organ shows a different color, a reddish or purplish tinge, which, however, disappears as the plant matures. The upper surface of the blade is a bright green, while the lower tends to take on a grayish tinge owing to the presence of hairs.

The root system of the cane, like that of other grasses, is composed of numerous fibrous roots. A distinct taproot is found only in seedlings and even here it is poorly developed, ceasing to grow after a brief period. The secondary lateral rootlets are much thinner than the primary ones and have but an ephemeral existence. When they become detached from the larger roots they leave abscission zones which may serve as infection courts for numerous parasites which, as is well known, invade the root system of the cane more readily than that of other plants.

The inflorescence forms a large open panicle. The flowers are arranged in small spikelets, each surrounded at the base by a tuft of silky hairs from two to three times the length of the spikelets. The latter are grouped in pairs: one sessile, the other pedicelled. Both spikelets are perfect and awnless. Each flower is subtended by two bracts which form the outer and the inner glumes. The outer glume is membranaceous, pointed, entire-margined and two-nerved. The inner glume is similar, but possesses a median keel. Continuing the two-ranked arrangement of the two glumes is the sterile lemma which is lanceolate, pointed, and practically without veins. The fertile lemma and palet of the typical grass flower are wanting. At the base of the flower, just inside the inner glume, are two thick, hyaline lodicules. These two lodicules and a small scale just inside of and inclosed by the sterile lemma may be considered the trimerous perianth of the flower. Lastly, the axis bears a whorl of three stamens and the ovary. The stamens have two-celled, versatile anthers, each suspended by a delicate filament. The ovary is short-stalked, erect, one-celled, and contains one ascending anatropous ovule. Owing to infertile or degenerate pollen in the Louisiana Purple variety, viable seeds are born only when foreign pollen is introduced.

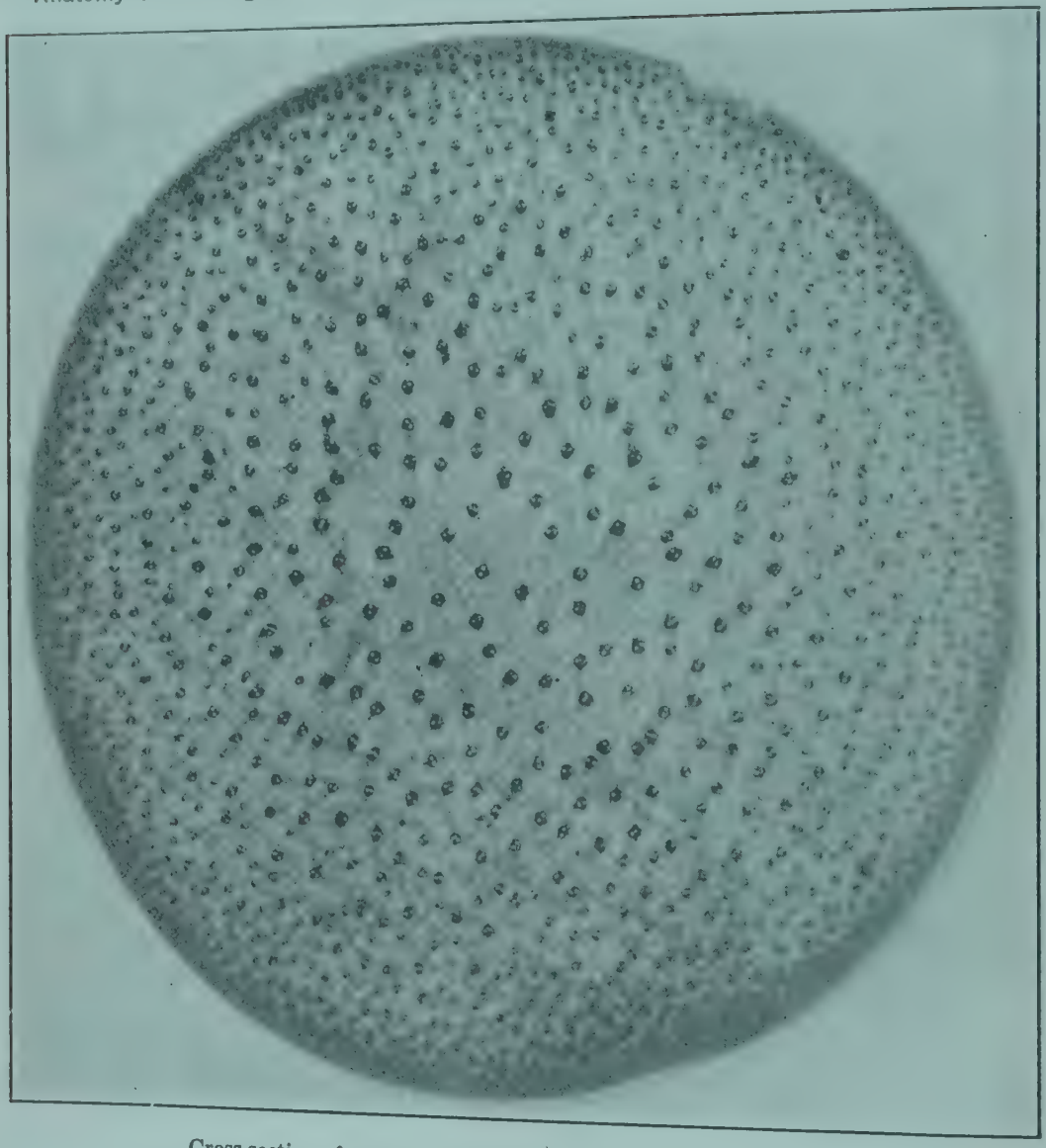
The fruit is small, about 1½ mm. long with a distinct constriction in the region opposite the embryo. It is indehiscent and included in the glumes and the sterile lemma. The pericarp is thin and intimately united with the testa. The comparatively large embryo lies in contact with the starchy endosperm by means of the cotyledon, which acts as an absorbing organ.

The sugar cane grows acropetally by a cone-shaped growing point. The increase in length of the stem, however, is due to an intercalary meristem at the base of each internode. The leaves are cut off by the apical growing point in rapid succession, and as new leaves appear a bud is formed in the axils of the older ones. The leaves grow much more rapidly than the stem, as can easily be seen in secondary shoots which have leaves several feet long, while the growing point is still underground. The formation of successive leaves and the subsequent growth in the intercalary zone divides the stem into segments, each consisting of a node and an internode. After the plant has reached a certain size, the lower underground buds initiate development and form secondary shoots, the amplification of this process resulting in stools which may contain scores or even hundreds of stalks.

ANATOMY

The material for study was grown in greenhouses at the Arlington Experiment Farm, Rosslyn, Va. The greenhouses had been especially constructed for the growing of sugar cane, in order to insure the most natural development of the plants.

The material taken from the greenhouse was studied while fresh and many of the photographs were obtained from free-hand sections of fresh material. For the purpose of securing permanent records, representative material was killed in Flemming's stronger solution, embedded, some in paraffin, some in celloidin, sectioned, and stained in the usual manner.



Cross section of a mature internode (1,275 vascular bundles). × 3

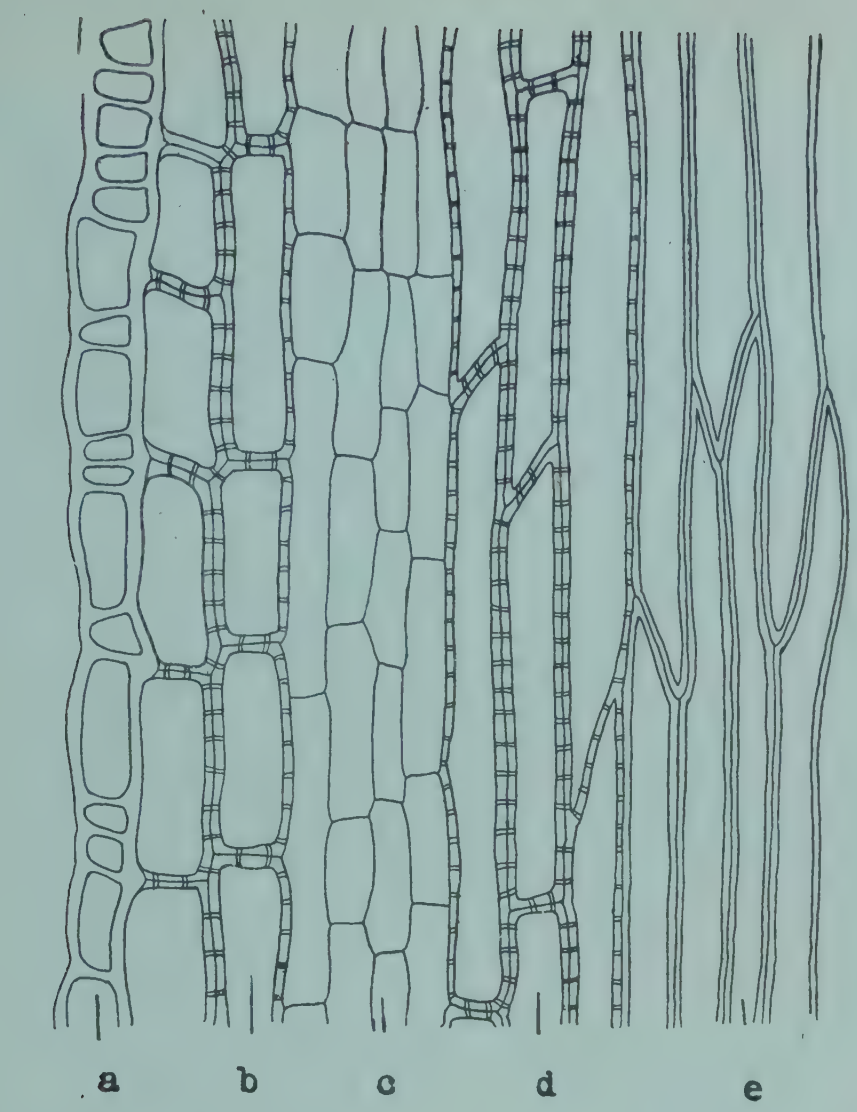
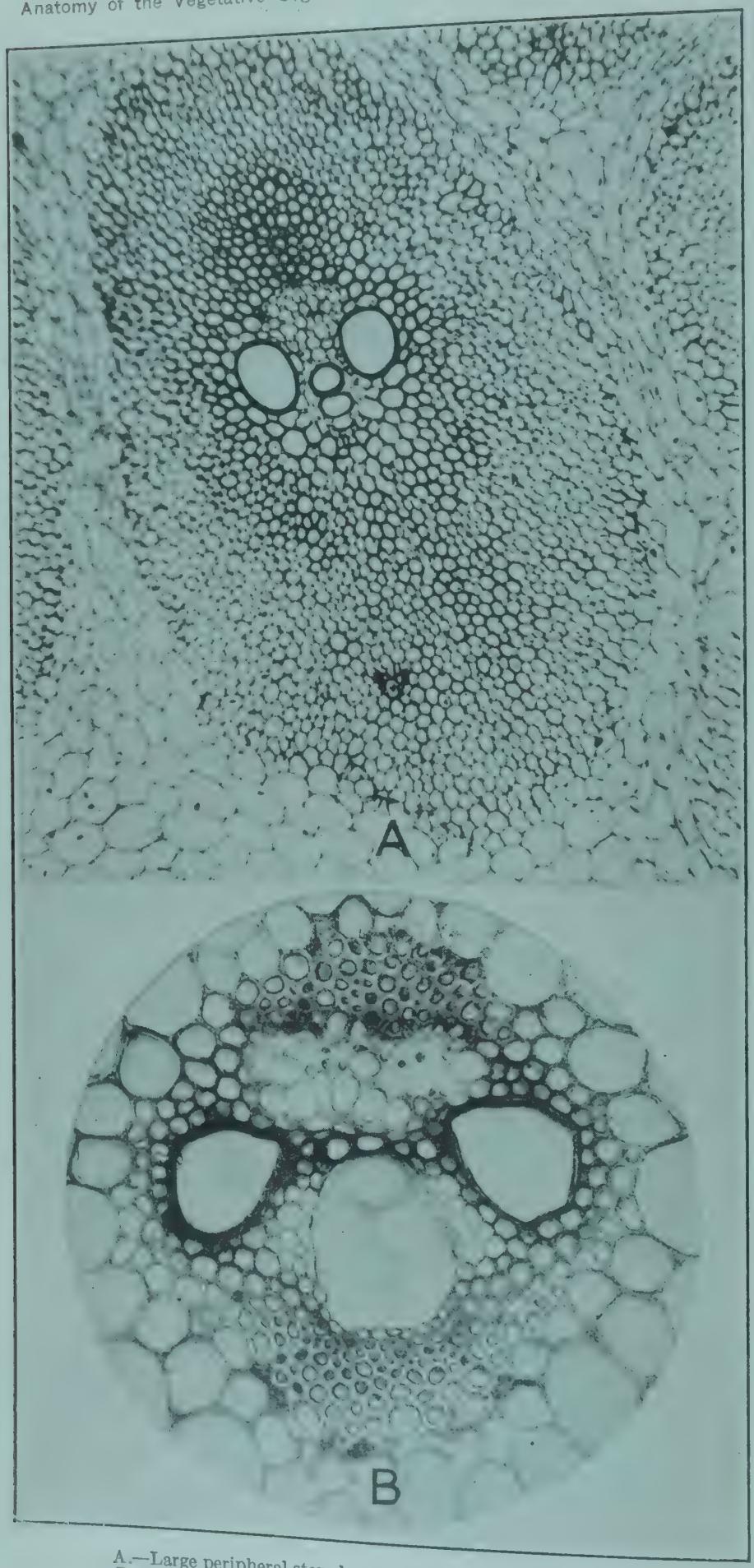


FIG. 2.—Longitudinal section through peripheral region of mature internode. × 360. *a*, Epidermis; *b* sclerenchymatous hypodermis; *c*, thin-walled cortical tissue; *d*, sclerenchymatous cortical tissue; *e*, sheath cells of peripheral stem bundles. Cross section through the nodal region of a mature stem. The epidermis is covered by a wax deposit in the form of densely crowded rods. The cells of the strongly lignified hypodermis have a large nucleus and cytoplasm. × 505.



A.—Large peripheral stem bundle. × 113
B.—Large bundle from the central region of the stem × 113

THE STEM

A node and an internode taken from the upper third of a large cane was used as representative material for the anatomical study of the stem. The internode was uniform, about 3½ cm. thick and 15 cm. long; the node was slightly thinner and exhibited regions of internal and external structural differences and will, therefore, in this discussion be subdivided into: (*a*) The node proper, which is limited above by the insertion of the leaf sheath; (*b*) the Keimring, which contains a bud and several rows of root primordia; (*c*) the intercalary meristem from which elongation of the internode takes place. These regions differ from each other, but because of the transition zones, however narrow, they will be treated in sequence rather than as altogether different entities.

A cross section of the internode (pl. 2) shows numerous vascular bundles embedded in parenchymatous tissue. Externally it is limited by a thick epidermis which, for further protection, is covered by a layer of wax. The vascular bundles are not arranged in a simple ring, but lie scattered throughout the section. Their number increases from the center toward the periphery, whereas their size gradually decreases. At the periphery the bundles are so small and so close together that they form practically a solid ring. The vascular tissue is separated from the epidermis by a cortex which varies in width and composition with different regions of the stem.

The parenchyma constitutes the filler between the bundles, except in the peripheral region, where it forms an uninterrupted layer comparable to the cortex of dicotyledonous plants. The parenchyma cells are thin-walled and separated by small intercellular spaces. In cross section the cells are roundish or somewhat elliptical, whereas vertically they have the form of short cylinders with straight or sometimes rounded ends. The depth of the cells varies with their position in the stem, those in the internode being elongated near the vascular bundles, but in the nodes the cells are very short regardless of their radial position.

The cortex forms a narrow band of tissue except in the Keimring where its width more than doubles. The two to four rows of cells next to the epidermis are small, thick-walled and lignified (pl. 15, A, and fig. 2). The vertical continuity of this sclerenchymatous mantel, however, is broken by the occasional interpolation of parenchymatous cells which abut externally on a stomate of the epidermis. This layer is followed by several rows of thin-walled parenchyma, the cells of which remain cellulose even in old and woody stems. There is a gradual transition from the cells of the cortex to those of the bundle parenchyma. The cells of this transition zone, which form the filler between the peripheral bundles, grow progressively larger centripetally and the walls become lignified wholly or in part. In the region of the Keimring the cortex is composed of uniformly small cells which remain cellulose. In very old stems, however, the two hypodermal layers may show partial lignification.

EPIDERMIS OF THE STEM.—An epidermis typically forms a single layer of cells, possesses stomates and produces outgrowths in the form of hairs. In the stem of the sugar cane, however, while stomates are sparingly developed, hairs are entirely wanting, though in a different variety they have been reported once by Soltwedel (*11, p. 28*).

The individual cells of the epidermis vary in size and form; on the whole, however, they are remarkably uniform. There are present two distinct types of cells alternating with one another; elongated rectangular cells with undulating walls, and short cells which occur singly or in pairs (pl. 5, C).

The long cells form four-sided prisms with a mean radial diameter of 9.8μ and a length varying between 54 and 184μ. The outer walls are greatly thickened and cuticularized. There is also present, especially in the region below the insertion of the leaf sheath, a wax deposit composed of a layer of densely crowded wax particles in the form of hooked rods (fig. 2). The walls of the epidermis are pierced by numerous pits, which are easily seen when the section is treated with chloral hydrate or some suitable clearing agent. In the region of the intercalary meristem the epidermal cells are very broad, and the walls less tortuous and comparatively thin (pl. 5, A).

The short cells are transversely rectangular, but the two components of the pair are rarely uniform. One is usually smaller, narrower tangentially and has silicified walls. The larger of the two has a tendency to be irregular; it is frequently pointed, which gives it the appearance of a wedge. Occasionally one or even both of the short cells are wanting, which results in the latter case in a continuous longitudinal row of long cells.

CENTRAL STEM BUNDLES.—In order to fully appreciate the different aspects

of the vascular bundles in the various regions of the stem, it seems best to take typical bundles, study their structure and note the changes that take place as these bundles pass through the nodal and internodal region of the stem.

The typical bundle, as found in the larger part of the cane, except in the peripheral region, is rhomboid in cross section. It is surrounded by a well-marked sclerenchymatous sheath which is most strongly developed on the inside and the outside of the bundle where it forms typical bundle caps (fig. 3). The vascular tissue of the bundle has xylem and phloem disposed collaterally in relation to each other. The protoxylem consists of annular and spiral elements. Below it is the protoxylem lacuna, a lysigenous cavity into which often project the remains of the first-formed annular vessels (pl. 3, B). Above and lateral to the protoxylem are two large vessels with medium long articulations which communicate by a single large pore. The vessels are surrounded by tangentially flattened parenchyma cells (pl. 4, A), which commonly possess reticulate thickenings. There is profuse pitting between the vessels and the parenchyma, but where a vessel directly adjoins the sheath

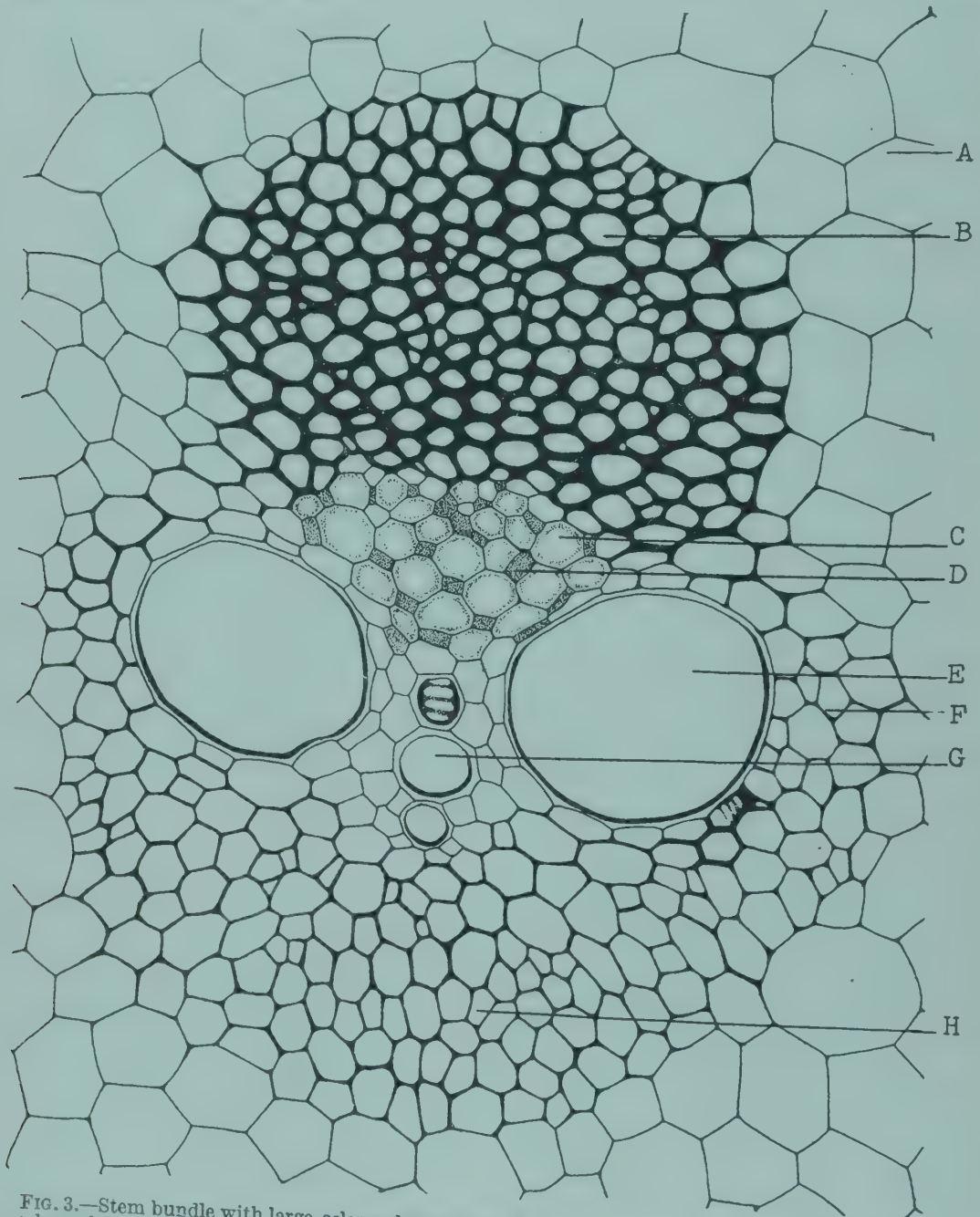
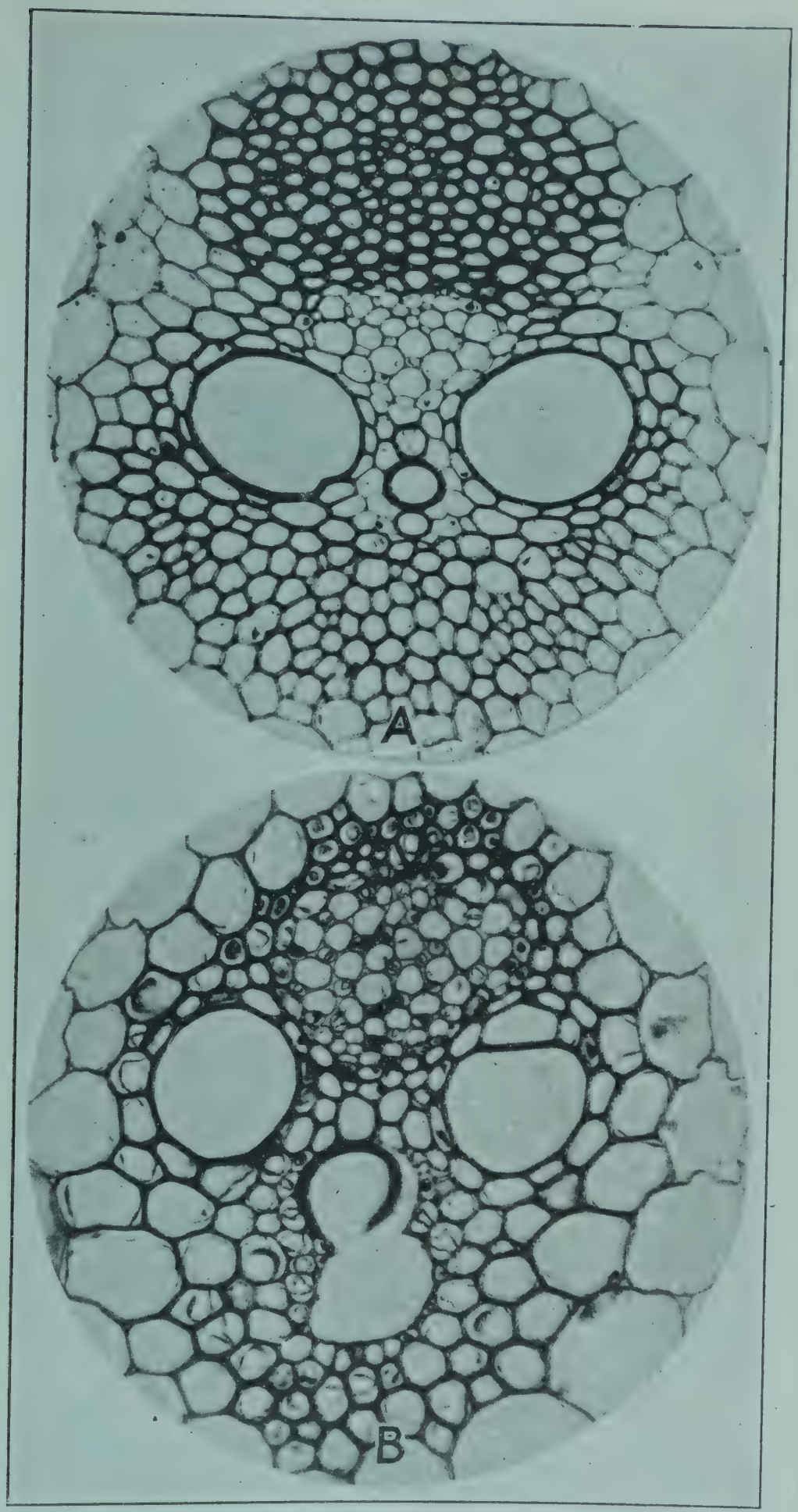


FIG. 3.—Stem bundle with large sclerenchyma cap. × 290. *A*, outer bundle parenchyma; *B*, sclerenchyma cap; *C*, sieve tube; *D*, companion cell; *E*, large pitted vessel; *F*, bundle sheath; *G*, protoxylem; *H*, sclerenchyma cap of xylem pole of bundle; it is poorly developed compared to the cap on the phloem pole.



A.—Large bundle from the more peripheral region of the internode. × 200
B.—Large bundle from a leaf sheath. × 350

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cells, the wall of the vessel next to the sheath cell is not pitted. The tissue between the two large vessels is composed of parenchyma interspersed with narrow vessels (pl. 3, B). The latter are porous and have reticulate thickenings on the side walls. The phloem forms an oval mass of tissue composed of sieve tubes and companion cells. The protophloem lies farthest to the outside; its walls are swollen and somewhat disorganized. This condition is, however, more strongly marked in the protophloem of the leaf (pl. 4, B), and in the stem bundles of Zea (pl. 25). The cells of the bundle sheath are in direct contact with the protophloem; the other phloem cells, however, are separated from the sheath by a layer of small, elongated parenchyma cells. A similar band of parenchyma, one or two cells wide, separates the phloem from the xylem.

The bundle sheath forms a layer of cells which are devoid of intercellular spaces. The cells are elongated, thick-walled and sparingly pitted. The end walls are pointed, except at the junction of xylem and phloem: here the sheath cells not only have a shorter vertical diameter, but the end walls are also nearly transverse.

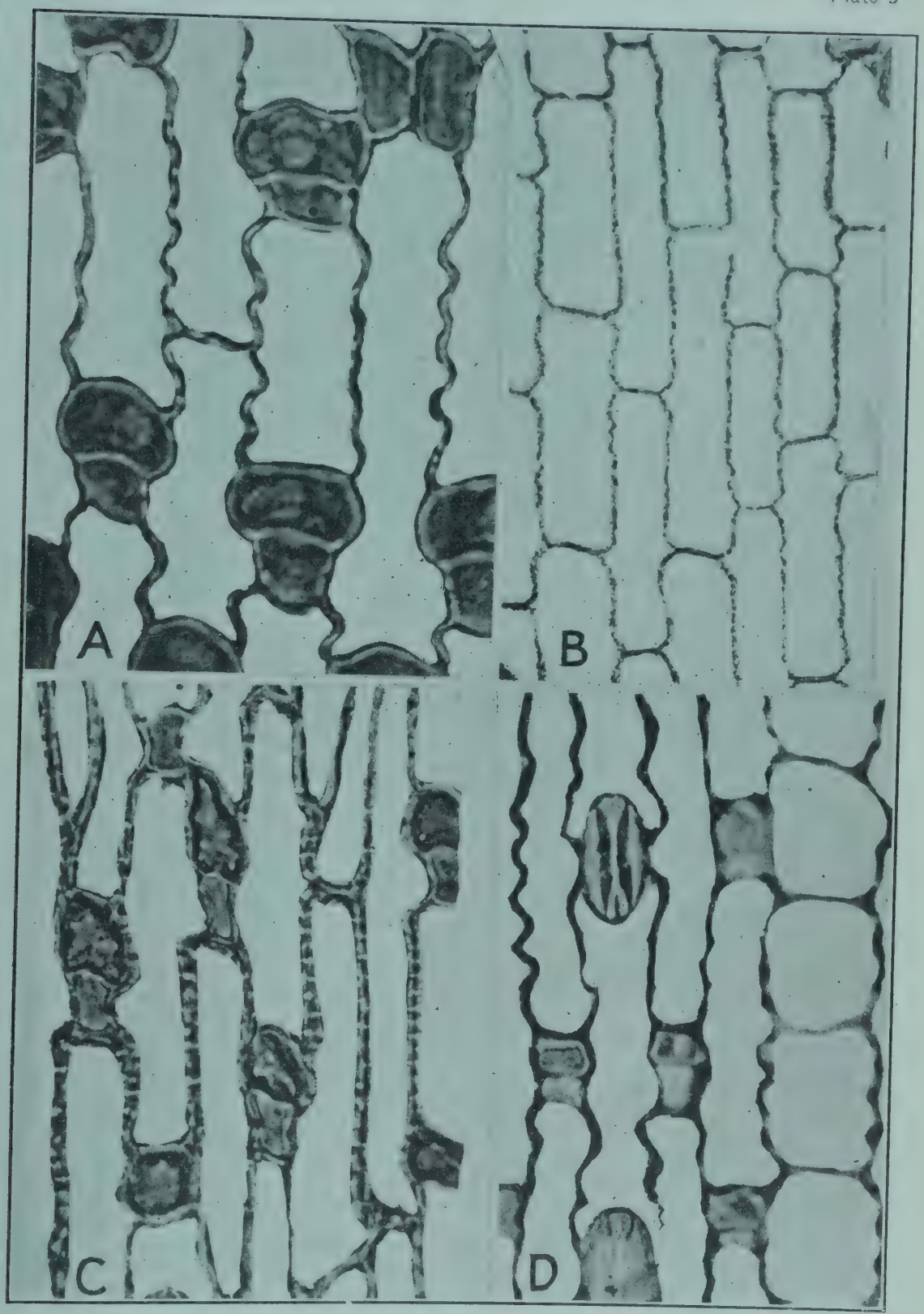
The bundles follow a longitudinal course approximately parallel to each other except in the node where a number of them branch or bend abruptly and move toward the periphery (pl. 1, A). Many of the bundles, however, pass on to the next internode with but slight deviation from their former course. Nevertheless the structure of all bundles is altered when they enter the node.

The peculiar appearance of the nodal bundles when viewed under low power is produced by the parenchyma adjoining the sheath. The parenchyma cells bordering the sheath are greatly enlarged radially and arranged around the bundle in stellate fashion (pl. 6, A). This same arrangement of the parenchyma is often found in the cells adjoining the protoxylem. The sclerenchyma cap of the phloem pole of the bundle is greatly enlarged; the walls of the cells are very thick and strongly lignified. The sclerenchyma cap of the xylem pole of the bundle, however, has practically disappeared. The cells of the bundle sheath in this region are lignified, but the walls have remained thin. The nodal bundles lack the protoxylem lacuna. There is a general increase in the number of protoxylem elements which extend upward and come in contact with the phloem. Occasionally the radial row of protoxylem cells is topped by a tangential band of annular or spiral elements, connecting the two pitted vessels. The latter also have a tendency to increase in number; the supernumerary cells, however, are smaller and less regular. The phloem shows on the whole an appreciable quantitative increase over that found in the internode. The phloem cells, however, have a tendency to become lignified (pl. 6, A), and nonfunctional. The extent of lignification varies, but seems to be in direct proportion to the size and age of the bundle.

As the vascular bundles leave the node and enter the Keimring, they change back to their old form. The stellate parenchyma disappears, the number of pitted vessels is reduced to two, and the protoxylem lacuna is formed again. Changes in the vascular sheath include the development of a cap on the xylem pole of the bundle and a reduction in the size of the cap on the phloem pole. The phloem shows little or no alteration. There is still a great deal of lignification, which is here sometimes more pronounced than in the nodal region.

Before the bundles definitely pass out into the next internode they traverse a region which yields more readily to the sectioning razor. This region is the intercalary meristem from which elongation of the internode takes place. The vascular bundles show surprisingly little lignification, except where the stem is so old that all meristematic tissue has completely matured. The chief divergence from the normal structure consists in a transformation of the sclerenchyma bundle caps into collenchyma and in a general reduction of the lignified cells of the vascular part of the bundle.

PERIPHERAL STEM BUNDLES.—Toward the periphery of the stem the bundles are more crowded, while their form changes from rhomboid to oval with the long diameter radial in respect to the stem (pl. 7, B). The outermost circle of vascular tissue is formed by small bundles which are almost spherical and practically form a solid ring at the periphery of the stem. The oval bundles are actually smaller than those nearer the center of the stem. The apparent increase in size is due to the development of a huge sclerenchyma sheath which is most prominent on the xylem pole of the bundle (pl. 3, A). The phloem is greatly reduced. Its form is no longer oval, with the long diameter in the tangent of the organ, but more or less triangular or trapezoidal, with the narrow part sunken



A.—Epidermis from intercalary region of stem. × 980
B.—Inner epidermis from leaf sheath. × 200
C.—Epidermis of mature stem. × 730
D.—Lower epidermis from leaf blade. × 350

For the sake of clearness the content of the long cells has been blocked out in the negative, so that in the print they appear white

between the large pitted vessels. The protoxylem lacuna has disappeared and the bundle contains thick-walled tracheidlike elements instead. The most peripheral bundles lack the protoxylem and the protophloem and consist largely of sclerenchyma.

In the region of the node these large elongated bundles lose their identity. They appear to break up into small bundles of various configurations, which, together with the peripheral bundles already present in the internode, form a comparatively broad zone of small vascular groups, which, like all bundles of the node, are surrounded by stellate parenchyma (pl. 7, A).

When leaving the nodal region the peripheral bundles become more uniform again. The bundles continue to show large lignified sheaths, but there is no longer that maze of bundle fusion and splitting found in the lower zone. In the upper part of the Keimring the bundles have practically attained the structure and arrangement they possessed in the internode.

Just as the center bundles become temporarily modified when passing through the intercalary meristem, so the peripheral bundles in this region exhibit considerable deviation from the normal structure. The large peripheral bundles upon leaving the root zone and passing through the meristem become so closely crowded that they appear like a honeycomb in which the walls of the comb represent the parenchyma which separates the bundles. The crowding of the bundles is the result of their increase in size, which is produced by the development of a huge collenchyma jacket around a small vascular nucleus (pl. 8). The lignified tissue is reduced to a few protoxylem cells. In the mature stem the same condition exists, except that in addition to the lignified vascular cells there is a thin-walled lignified vascular sheath around each bundle. The sheath is potentially present in the young bundles, but since the cells are delicate and of cellulose, they are not readily distinguished.

SPECIAL BUNDLE STRUCTURES.—At the point of insertion of the leaf sheath the node is traversed by longitudinal traces which, descending from the leaf, penetrate almost horizontally to the center of the stem and thence perpendicularly downward (pl. 15, B). In their horizontal extent the leaf traces deviate from the normal structure. This deviation is shown first in the arrangement of xylem and phloem, which, instead of being distinctly collateral, approaches the amphivasal type. Secondly, the deviation is one of structure. One notices at first glance that the large pitted vessels are wanting and that instead there is an abundance of narrow elements with short articulations and secondary thickenings in the form of very close spirals. In cross section the bundle is typically heart-shaped (pls. 9, A and 10). The xylem is limited to the periphery and distributed in a manner that the narrow spiral elements occupy the tip of the bundle, while at the flanges are found lignified parenchyma cells with scalariform, sometimes reticulate, wall thickenings. The bundle sheath is indistinct and is composed of thin-walled, short fibers of large diameter. The phloem is abundant but lacks the characteristic structure produced by the relative arrangement of sieve tubes and companion cells. It is composed of nearly uniform polyhedral cells, many of which are sieve tubes. The elements of the protoxylem are embedded in parenchymatous tissue, the cells of which later enlarge and partially disintegrate, so that in the mature bundle there is commonly seen a large-celled and lignified lacunate tissue adjacent to the protoxylem.

In addition to these large leaf trace bundles there are numerous small ones which run at right angles to the stem axis. They often surround the ascending leaf traces (pl. 11, A), and frequently fuse with them. The phloem of these small bundles is relatively well developed, while the xylem is often reduced to a single large vessel (pl. 9, B). The bundle is surrounded by a sheath, the cells of which become lignified.

The internodes of the mature stem occasionally contain bundles which deviate from the common structure by the absence of the protoxylem and the reduction of the metaxylem to a single large vessel. The failure of the protoxylem to develop is due to the fact that the bundles were differentiated after elongation in the internode had practically ceased. A second type of deviation is caused by inversed orientation. It is not infrequent to find bundles, especially near the periphery, which have the phloem developed in the direction of the center of the stem, whereas the adjacent bundles are oriented normally, that is, the phloem facing the periphery.

RELATIONSHIP OF THE LEAF TRACES.—The large number of traces entering a leaf and the very complex nodal structure make it impossible to follow the course of the vascular

bundles, except in the meristematic region of the stem apex. Longitudinal sections through such a region show numerous bundles passing from the stem into the leaves. The largest of these bundles pass gradually from the periphery of the stem to the center, and from here, in a curve with the convexity upward, out into the leaf (pl. 12, A and B). The smaller bundles penetrate less deeply, and the smallest remain near the periphery. In regard to the length of the individual leaf traces the same sequence is observed, that is, the largest bundles are also the longest and pass independently through about eight internodes; whereas the smaller bundles are correspondingly shorter, and the smallest cortical bundles terminate in the same internode. Strasburger (*16, p. 328*) found in Zea that the longest leaf traces pass through six internodes. In the sugar cane, the writer has followed a number of the large traces through seven and even eight internodes; their absolute length, however, he was unable to determine with certainty.

The course of the leaf traces is still further complicated in that they describe a spiral in their passage through the stem. For this reason it is very difficult to follow the entire length of a trace in longitudinal sections. This peculiar course of the traces is also responsible for the crossing of traces, which is so noticeable in radial sections (pl. 12, B). The traces of the higher leaves will cross those going to lower leaves and the crossing will be the more striking the thicker the stem and the closer the leaves. In the mature stem, as has been noted, the bundles run approximately parallel to each other, but this is accounted for by the fact that the length of the internodes has increased extremely, and since the large traces pass through many internodes the seemingly parallel course of the leaf traces closely approximates the real situation.

Further complications of the course of the vascular bundles are seen in the node in connection with the vascular supply of the roots and the axillary bud. But, whereas the root connections are derived mainly from the peripheral zone of bundles, the numerous traces going to the bud anastomose with the bundle system of the entire cross section of the stem.

This net of horizontal traces (pl. 11, A) may serve in part other purposes besides the connection of the buds and roots with the vascular arteries of the stem and the leaves. Since there is located above the Keimring the intercalary meristem, where active growth takes place, these numerous horizontal bundles may serve to conduct food material to this meristematic zone, as suggested by Haberland (*7, p. 338*).

THE LEAF SHEATH

A cross section through the middle of the sheath, about midway between sheath and blade joint, shows radial rows of vascular bundles, embedded in fundamental parenchyma (pl. 13, D). The cells of the latter, however, are mostly broken down, so that the vascular bundles are actually separated radially by large air cavities. The vascular bundles lie closer to the outer epidermis (morphologically lower surface) than to the inner one (pl. 14, D). There are bundles of two or sometimes more different orders of size alternating with one another. The large ones occupy approximately the center of the sheath in a cross section, while the smaller ones are close to the outer epidermis and may even touch it. The large bundles frequently consist of two or even three superimposed groups (fig. 4) connected by bands or caps of sclerenchyma. They have the typical shape described for the bundles of the stem, except that in the sheath the phloem is more strongly developed (pl. 4, B). The entire bundle is surrounded by a sclerenchyma jacket which is a continuation of the sheath of the stem bundles. The smallest bundles, which always lie closest to the outer epidermis, consist largely of a mass of sclerenchyma inclosing a few vascular elements.

The parenchyma cells which form the radial prolongation of the bundles in the direction of the inner epidermis (pl. 15, E), show a more or less definite arrangement and terminate in a small mass of sclerenchyma (pl. 14, D), which is separated from the inner epidermis by a row of narrow, parenchymatous cells.

The base of the sheath, also called the sheath-joint (pl. 1 B and pl. 13, A), is swollen and lacks the projecting veins of the sheath proper. Structurally it reflects the molding influence of its location which demands great elasticity of the tissues coupled with late maturation. Increase of flexibility is attained by transformation of the sclerenchymatous elements of the bundle into collenchyma, while greater strength is at the same time afforded by the disappearance of the large air cavities, and the development of small-

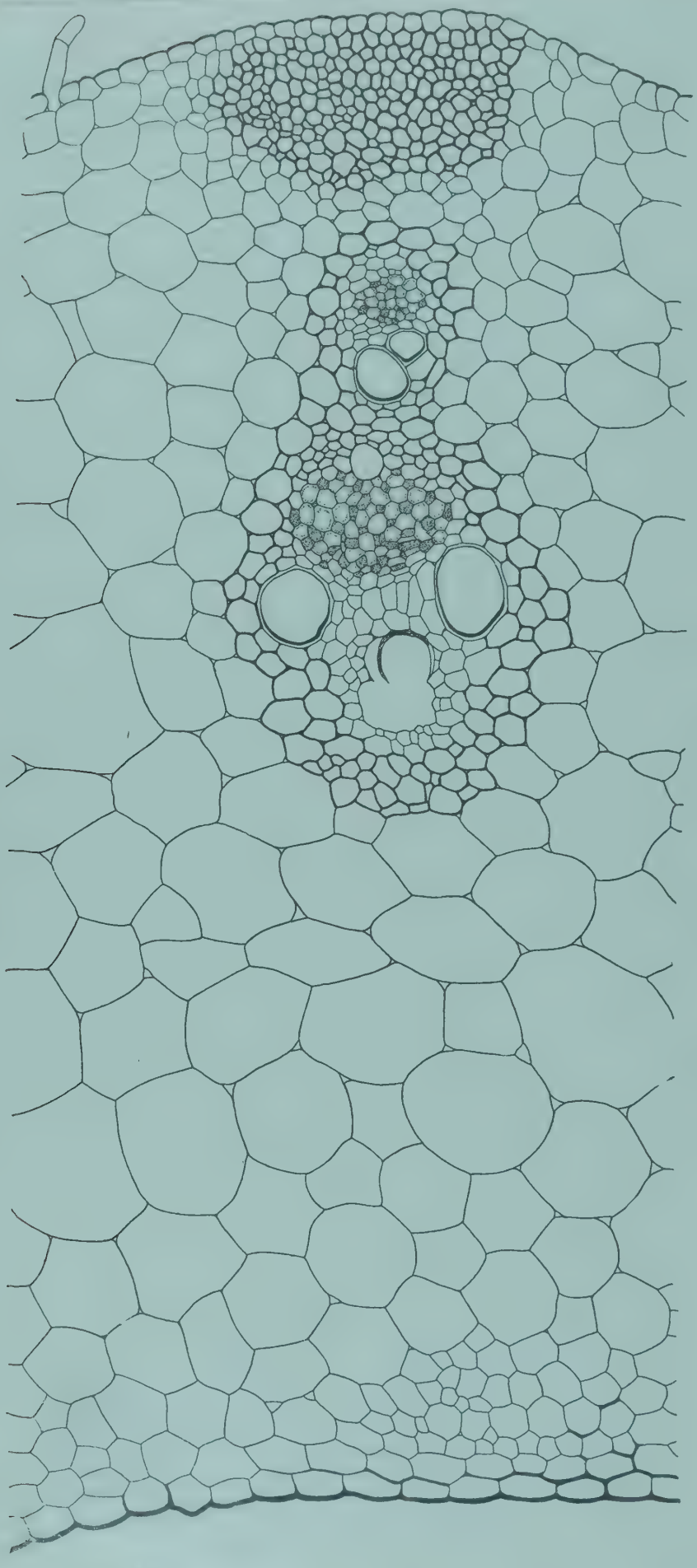


FIG. 4.—Cross section through leaf sheath. ×200

celled parenchyma instead. A cross section through the sheath joint just above the insertion on the stem (pl. 15, D), shows an increase in the number of bundles over that of the sheath proper, and their partial radial displacement. As a result of these changes, no longer are there rows of superimposed bundles, but more or less definite tangential bands, with each band containing bundles of a different magnitude. The bundles of the inner band, that is, the ones closest to the inner epidermis, are the largest and most widely spaced, and are often compound. They constitute the large leaf-traces which, as may be seen from a study of the course of the vascular bundles, penetrate far into the stem and extend to a depth of several internodes. Often, just before passing out into the leaf, two or even more bundles fuse more or less completely, which accounts for the presence of the compound bundles in the sheath joint. The second circle contains smaller bundles which penetrate less deeply and are much shorter in vertical extent. Finally, the outer circle is formed by small groups consisting mostly of collenchyma (pl. 13, E).

The structure of the large bundles resembles somewhat that of the bundles found in the intercalary zone of the stem. On the xylem pole of the bundle there is a large collenchyma cap which is separated from the bundle itself by a single layer of parenchyma. The xylem is composed of spiral elements which partly surround the phloem. The smaller bundles have increasingly larger amounts of collenchyma, and the smallest may be exclusively collenchyma. Both below and above the sheath joint the collenchyma terminates and is replaced by sclerenchyma. The supernumerary bundles gradually disappear, and between the radial rows of bundles the parenchyma exhibits partial disintegration with the formation of the typical air cavities.

In the growing cane, the lower, older leaves are progressively shed, constituting the so-called process of "self cleaning" typical of this group of cane varieties. In the mature plant there is a naked stalk with a tuft of large leaves at the apex. The leaves become detached from the stem in the region of the sheath joint, which, as previously discussed, is anatomically different from the other regions of the leaf, in that the sclerenchyma fibers are replaced by the much softer collenchyma. The sheath joint may in this connection be considered the abscission zone of the leaf.

The apex of the sheath also undergoes modifications before it merges into the blade. As the leaf sheath approaches the blade joint it becomes narrower and thicker, thereby crowding the vascular bundles more and more together. The air cavities between the bundles disappear, being replaced by parenchymatous tissue. The sclerenchyma groups near the outer epidermis gradually enlarge, while the vascular bundles at the same time move closer to the center of the sheath. The sclerenchyma groups of the inner epidermis also enlarge considerably and gradually unite into a solid tangential band (pl. 15, C). While these changes are going on, the tangential band of sclerenchyma becomes separated from the inner epidermis by a progressively widening band of parenchyma (pl. 15, C).

The part of the sheath just above the insertion of the ligule has been referred to as the blade joint (pl. 1, C and fig. 5). The flanges of this joint are brownish in color, strongly pubescent, and of a soft texture. The vascular tissue in this region resembles that found in the sheath joint. As in the latter the large bundles inclose much collenchyma, whereas the xylem consists entirely of spiral vessels. The vascular tissue of the median blade joint, which becomes continuous with the bundles of the midrib of the blade, does not show the modifications exhibited by the flanges. If there are any noticeable changes they are in the direction of increased lignification and the formation of ever broadening lignified bundle caps. In the transition region from the median part of the blade joint to the flanges, there is noticeable a gradual transformation of the sclerenchyma into collenchyma; one frequently observes bundles which have thick collenchyma caps, of which half of the cells consist of lignified sclerenchyma.

The inner epidermis of the sheath differs fundamentally from the outer epidermis in that the cells of the former are large, uniformly rectangular, and of one type, since the short silicified cells are wanting (pl. 5, B). Hairs and stomates are only sparingly developed, except just above the insertion point of the ligule, where they form a dense fringe. This same type of epidermal cell is found on the inner surface of the ligule (the side which is opposite the stem). The cells just below the ligule are broad and more or less irregular, whereas above the ligule they show a transition to the cells of the leaf blade, and become long, narrow, and thick walled.

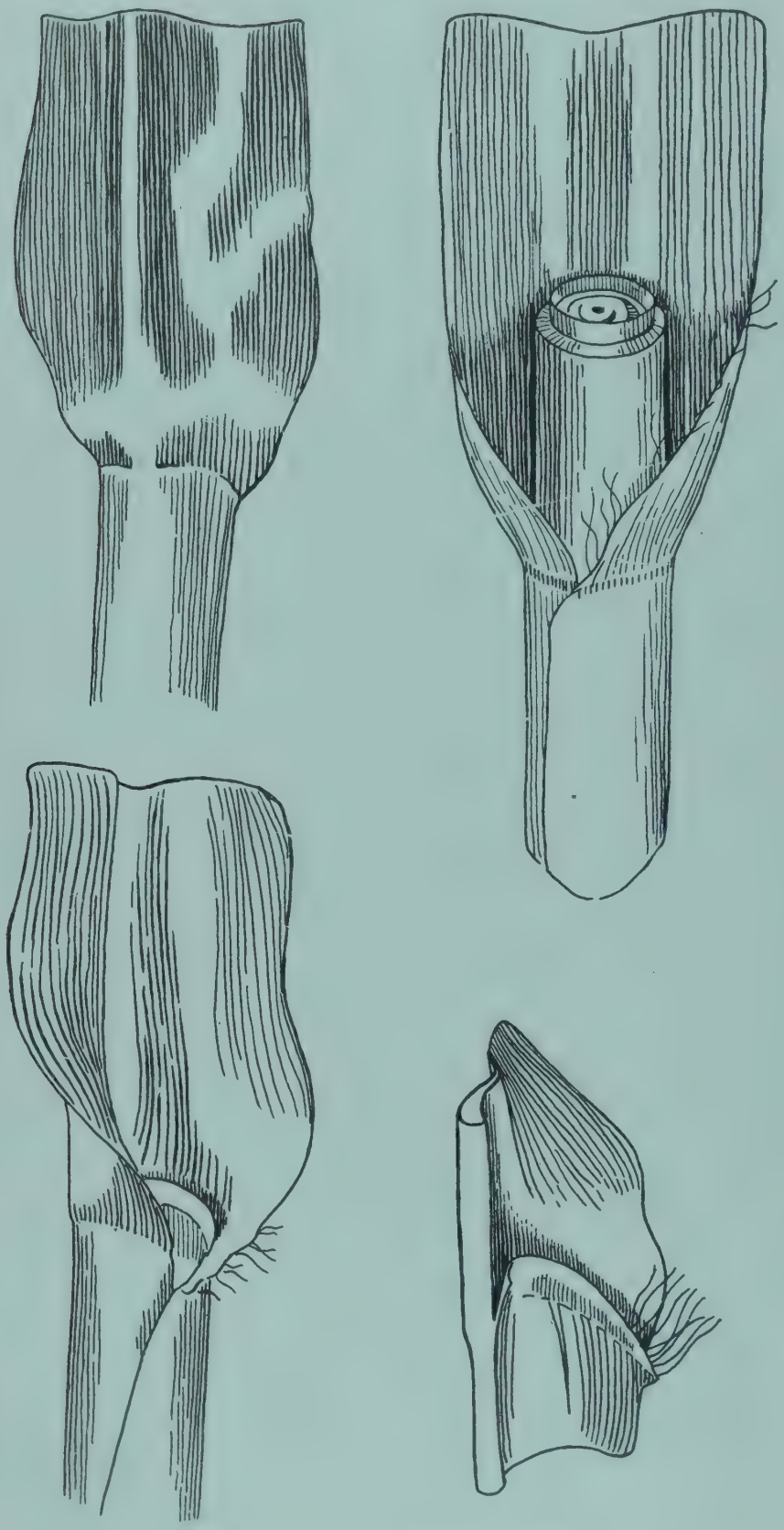


FIG. 5.—Four views of blade joint and ligule

The cells of the outer epidermis of the sheath resemble those of the stem (pl. 13, C). They are very thick-walled, undulated and pitted. The short cells are very conspicuous and in specialized regions, as for example at the blade joint, they are more numerous than elsewhere. The cells above the veins are always long and narrow and possess a nearly closed lumen; those between the veins are broader, but otherwise structurally the same. The epidermal cells of the sheath joint and the flanges of the blade joint are thin-walled and irregular in shape. Stomates and hairs are well developed. The former occur in longitudinal rows next to the veins. The hairy covering is most extensive above the sheath joint (pl. 14, B) and at the flanges of the blade joint. Above the sheath joint the number of hairs decreases greatly, coincident with a change in their character. Instead of the extremely long and delicate hairs which are characteristic of the regions referred to above, short and strongly lignified setae interspersed with two-celled appressed hairs are found.

THE LIGULE

The ligule contains no vascular tissue but is made up altogether of elongated parenchyma cells which are comparatively thin-walled and of cellulose. Both inner and outer epidermis are suberized. The outer epidermis, the one next to the sheath, is covered with numerous long hairs which, arising at the base of the ligule, are adnate to the epidermis (pl. 13, C) and become free at the apex where they form a minute fringe. The hairs are commonly strongly lignified at the base. The outer epidermal cells are fairly thick-walled and slightly undulated; the inner ones are broad, especially at the base, the walls are thin and more undulate than those of the outer epidermis. All epidermal cells are of one type, silicified cells as well as stomates being absent. The ligule has a protective function in that it prevents the entrance of water between stem and sheath.

THE LEAF BLADE

The tissues of the blade are continuous with those of the sheath and in general show similar structure. Differences such as are found are consistent with the chief rôle of the blade as an organ for assimilation and aeration.

The vascular bundles are of three ranks (pl. 16, C): (*a*) small round bundles, (*b*) medium large oval bundles, and (*c*) large rhomboid bundles. The small and medium large bundles alternate with one another, whereas the large bundles, corresponding with the more prominent veins of the leaf, appear at wider intervals. The large bundles are always bound on either side by a small round bundle. The small bundles are always close to the lower epidermis, whereas the other two types occupy the middle of the blade. In the region of the midrib (pl. 16, B) however, a layer of colorless parenchyma is interpolated between the bundles and the upper epidermis. This layer increases in thickness, pressing the vascular tissue gradually toward the lower surface, while the scattered sclerenchyma groups on the upper surface unite to form a solid band. The resulting tissue area has the form of a half moon, or triangle, the lower surface of which is studded with vascular bundles, while the upper surface, which contains no bundles, is reinforced by a solid layer of sclerenchyma.

The bundles of the blade differ primarily from those of the stem in that they possess a chlorophyll-bearing bundle sheath and an inner sheath in the region of the phloem (pl. 1, D). The vascular tissue itself differs in the lack of a protoxylem lacuna except in the very large veins, and in a greater development of the xylem (pl. 17, A).

The chlorophyll-bearing bundle sheath, which forms a continuation of the sclerenchyma sheath of the stem and leaf sheath bundles (pl. 17, B), is made up of uniform large and spherical cells which vertically have the form of short cylinders (pl. 18, B, C). In the region of the phloem pole of the large bundles the sheath cells become smaller or may even be wanting. Through the gap, which is formed thereby, the fibers of the inner sheath become continuous with the fibers of the hypodermal sclerenchyma. But while the fibers of the inner sheath appear, in some instances at least, ontogenetically the same as those of the hypodermal sclerenchyma, they differ structurally in that the former possess numerous pits. In small bundles, where the cells of the inner sheath are undifferentiated as yet, the phloem is separated from the chlorophyll-bearing sheath by a layer of parenchyma cells, which differ from the phloem cells only by their larger size.

The xylem of the large bundles consists of two large vessels connected by a more or less extensive band of small pitted elements, most of which are narrow vessels. In the small bundles there are only a few rela-

tively large pitted vessels which border directly on the phloem (pl. 17, A.) As is usual, however, phloem and xylem cells do not communicate by pits. The phloem is structurally similar to that found in the bundles of the leaf sheath. This is especially true of the bundles of the midrib (pl. 18, A). The sclerenchyma cap of the bundles of the midrib abuts directly on the epidermis.

Transverse bundle connections are frequent. They are in the nature of narrow branches which run somewhat diagonally, or at right angles, between two veins and effect union with the latter. As the leaf becomes narrow there is a gradual reduction in the number of bundles. The bundles which are to drop out either fuse with other bundles or they become so completely reduced that only a few phloem and xylem cells remain and finally only elongated parenchyma.

The chlorophyll-bearing parenchyma of the leaf forms a more or less concentric ring around the bundles, except when the latter are so large that they practically fill the area between the two epidermis. In the midrib the chlorophyll-bearing parenchyma is found largely between the small bundles. The cells are small, irregular, and filled with numerous chloroplasts, the color of which is darker green than that of the plastids found in the sheath cells. This apparent difference in color is probably due to the fact that in the spongy parenchyma the chloroplasts are more crowded than in the sheath where they are few in number and much larger individually. In fixed material the large chloroplasts have a tendency to clump together in narrow crescent-shaped bands, (pl. 17, A). Adjacent to and partly surrounding the spongy parenchyma are large colorless cells, which have some peripheral protoplasm and a watery sap. They probably serve for the storage of water.

The cells of the epidermis are brick-shaped with the largest diameter in the direction of the long axis of the organ. The walls of the cells are strongly undulated, thickened and lignified (pl. 18, D). In addition to the long cells there are found, just as in the epidermis of the stem and leaf sheath, short cells singly or in pairs (pl. 5, D). One component of the pair is commonly thick-walled and strongly silicified. The epidermal cells above the veins differ from the normal type in being greatly elongated and often so thick-walled that there is hardly any lumen. The upper epidermis has in addition to the two types of cells mentioned, the so-called "bulliform" cells (pl. 16, A), which form longitudinal bands and alternate with strips of ordinary epidermal cells. The bulliform cells are much larger than the other cells of the epidermis and differ further in that their walls remain thin and of cellulose. They are continuous with the inner part of the leaf through the large colorless cells referred to above. The bulliform cells differentiate only after the leaf begins to unroll, but then they increase in size rapidly and become slightly raised above the level of the other cells.

The margin of the leaf is serrate; the teeth are formed by stiff setae of somewhat triangular form. The rows of epidermal cells next to the margin are thick-walled, colorless, and almost devoid of content.

Except for the structural deviation exhibited by the margin of the leaf the lamina is remarkably uniform. The veins, which occur at regular intervals, divide the blade into narrow bands of tissue composed of rows of regular epidermal cells, alternating with rows bearing either stomates of hairs. The latter exhibit two distinct types: short one-celled bristles, which attain a larger size at the lower leaf surface, and two-celled appressed hairs. There are commonly three rows of stomates between two veins, but on the upper leaf surface they are less numerous and are commonly limited to a single row at each side of a large vein.

The stomates are phaneroporous, lying in the same level with the epidermal cells. They are always found in longitudinal rows in such a manner that a stomate alternates with an epidermal cell. The mature stomate is formed by two guard cells and the adjacent subsidiary cells (pl. 19, A, C, D, and E). The latter become much larger than the guard cells, extend deeper radially and their outer walls slope slightly in the direction of the guard cells. The guard cells are wider at the ends than in the middle region of the cell. In longitudinal sections it will also be noticed that both tangential walls of the guard cells, but especially the inner walls, are deeply constricted in the middle, which further tends to narrow the lumen of this part of the cell. The pore of the stomate is elongated lozenge-shaped, its lateral walls being straight and parallel, or sometimes gently curved. The stomatal movements are correlated with this peculiar construction of the guard cells. Schwendener

(*15*), who fully investigated the stomatal movements of the grasses, explains the opening and closing as follows: Since the middle portion of the guard cell is narrow and the tangential walls greatly thickened, any change in the osmotic pressure of the cell would be incapable of causing movement in this portion of the wall. The enlarged ends of the guard cells, however, have thin walls which will respond to turgor changes. When there is an increase in cell turgor they cause the pore to open, whereupon the thickened middle portions of the guard cells are passively drawn apart leaving a slit with parallel sides.

THE ROOT

The root differs from the stem by two structural features, the possession of a root cap, and the radial arrangement of the bundles. The root cap occupies the extreme tip of the root. It is often colored red, which, ordinarily a normal condition, sometimes is of pathological significance.

A cross section of a young root (pl. 20, A) shows a cylinder of vascular tissue limited on the inside by a well developed pith, on the outside, by the endodermis and cortex. The pith is of uniform nature and is made up of large spherical cells, interspersed with small intercellular spaces. The cells are elongated vertically and have a tendency to become larger toward the center. Occasionally there are found scattered in the pith small, thick-walled cells similar in size and shape to the interstitial tissue of the stele (pl. 21, A). The cortex possesses on the inner and outer periphery, layers of small and specialized cells. Those at the outer periphery are narrow, elongated and thick-walled and form in their entirety a distinct sclerenchymatous cylinder. The rows of cells at the inner periphery consist of regular small square cells between which are found rhomboid intercellular spaces (pl. 20, B). The larger part of the cortex consists of loosely packed parenchyma cells which later disintegrate with the formation of large air chambers.

The epidermis is made up of fairly uniform and thin-walled cells which vertically have the form of short cylinders. Many of the epidermal cells have grown out into hairs which remain attached to the root long after they have ceased to function. Beneath the epidermis is the exodermis, a layer of thin-walled parenchymatous cells, which are larger and more elongated than the cells of the epidermis. The walls of these cells are suberized, and their inner tangential surface is always thickened.

The endodermis as shown in Plate 22, B, C, and D consists of a single layer of cells connected uninterruptedly with one another. The individual cell is a vertically elongated four-sided prism with horizontal ends. The radial walls slightly exceed in length the tangential ones, though sometimes they are equal. The cells are thin-walled in their meristematic condition, but upon maturing the inner tangential walls and parts of the radial ones become greatly thickened and lignified. The Casparian strips are seen in young material but even here only with difficulty; later they become masked by the development of secondary wall thickenings. The inner tangential walls of the epidermal cells are distinctly pitted and possess peculiar protuberances which extend into the lumen of the cells. The apical region of such protuberances as shown by Klinge (*9*) and Bremekamp (*3*) contains silica.

The vascular tissue consists of alternating masses of primary xylem and phloem which occupy different radii (pl. 23, A). The xylem plates do not reach the center. The latter is occupied by a parenchymatous pith at the periphery of which there is a ring of large pitted vessels. (Pl. 20, A and pl. 22, A.) Outside of this there is a ring of alternating xylem and phloem groups. In young roots (pl. 24, A) there are almost always eight large vessels; in older and thicker roots (pl. 21, A) this number is much larger. The clusters of protoxylem and phloem are very numerous (pl. 23, A), a result of the absence of secondary growth which is so important a factor in the development of the roots of the dicots. In the thin lateral roots and in the rootlets of seedlings, the xylem plates are few in number and meet in the center; a pith and the large pitted vessels are often wanting (pl. 24, B). The groups of xylem are separated from the endodermis by a single-layered pericycle, but in the small roots the xylem cells occasionally border directly on the endodermis. Between the groups of primary xylem and phloem is interstitial parenchyma (pl. 23, B and fig. 6). Cells of the same tissue also separate the protoxylem and phloem from the large vessels, then surround the latter and form a peripheral sheath around the pith. The character of these cells, however, differs with the region where they occur. Thus the

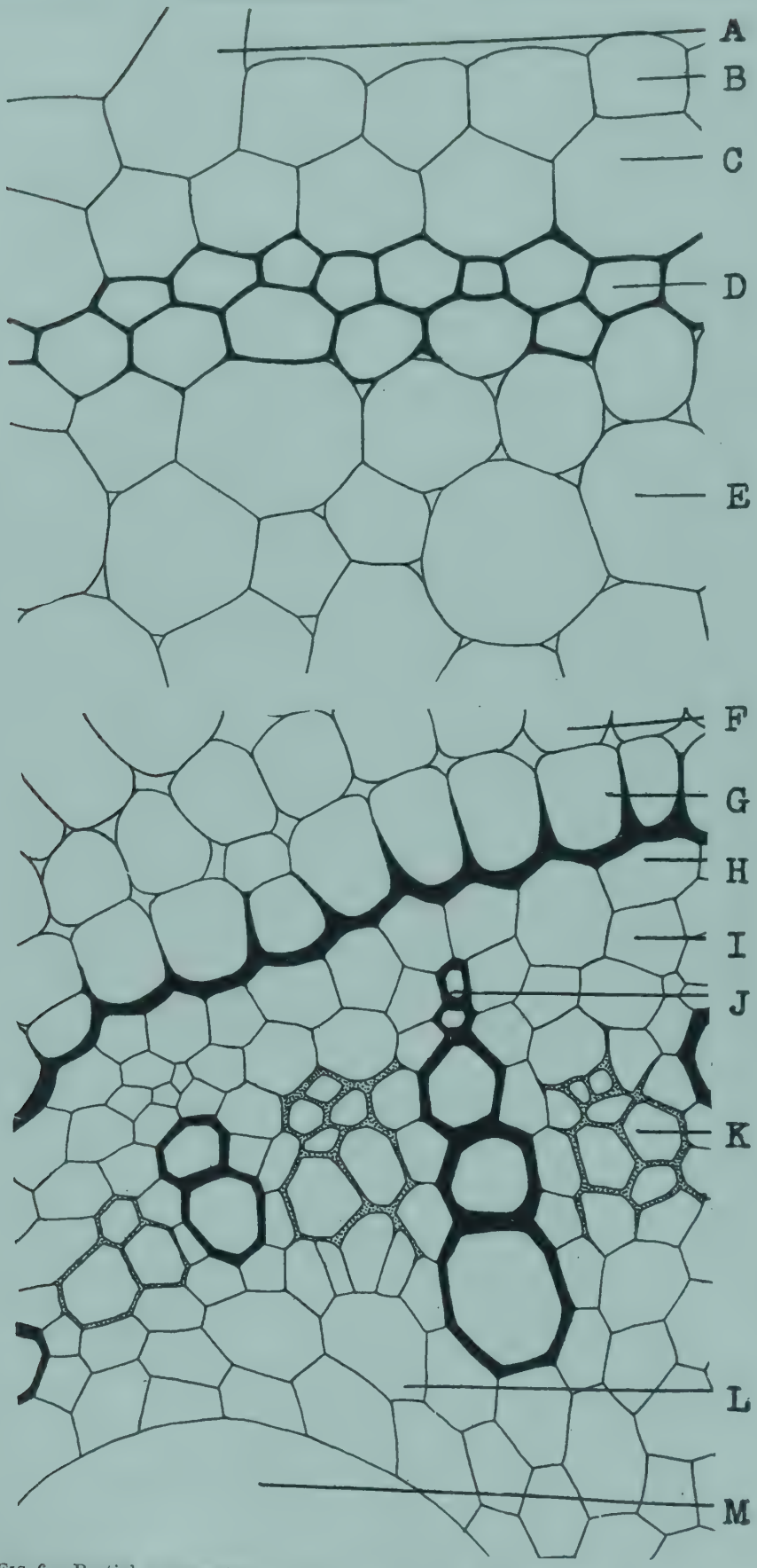


FIG. 6.—Partial cross section of young root. × 510. A, root hair; B, epidermis; C, exodermis; D, sclerenchyma tissue; E, cortex (only a few cell layers have been drawn—the actual cortex is broader); F, inner cortex; G, endodermis; H, pericycle; I, interstitial parenchyma; J, protoxylem; K, phloem group; L, parenchyma between protoxylem and large pitted vessel; M, large pitted vessel (still in meristematic condition)

cells which separate the groups of protoxylem are elongated, rectangular, and profusely pitted; those at the periphery of the pith are sclerenchymatous pointed, and sparingly covered with narrow slanting pits. The thickening and lignification of these sclerenchymatous cells begin soon after the protoxylem is formed and while the large vessels are still meristematic. The first of these cells to become lignified are those adjacent to the pit and the process of maturing is thence continued in centrifugal direction (pl. 20, B, and pl. 23, A). The order of development differs therein from that of the protoxylem, which is in the direction of the root axis.

The structure of the protoxylem of the root is peculiar in that it lacks annular and spiral elements. The first-formed cell is a narrow element with scalariform or spiral-reticulate wall thickening; the second protoxylem cell is similar though often much larger than the first one and is usually scalariform. Quite frequently the larger of the two protoxylem cells develops first and only later a small cells is added centrifugally.

The groups of phloem alternate with the protoxylem. They are always small and rarely contain more than eight elements. The cells of the phloem are pentagonal or square in cross section and are readily recognized by the slightly thickened refractive walls (pl. 23, B).

In old roots all the cells of the vascular tissue, with the exception of the phloem, become lignified and thick-walled. Similar changes take place in the layer of cortical cells which border on the endodermis. Concomitant with these changes is a disintegration of the cortical tissue. The radial strips of tissue which remain are attached to the exodermis on the one hand and the sclerotic layer of inner cortical cells on the other (pl. 21, B). Occasionally certain cells of the inner cortex do not lignify so that the radial strips of cortex become attached to the endodermis directly.

ONTOGENY

Material for study was selected from the apical region of a large, actively growing cane. The stem portion measured 9 cm. and contained 14 internodes of which the upper 10 were crowded into a space of only 10 mm. (fig. 7). The relative length of the leaf sheath and blade of the first 14 leaves follows on page 218.

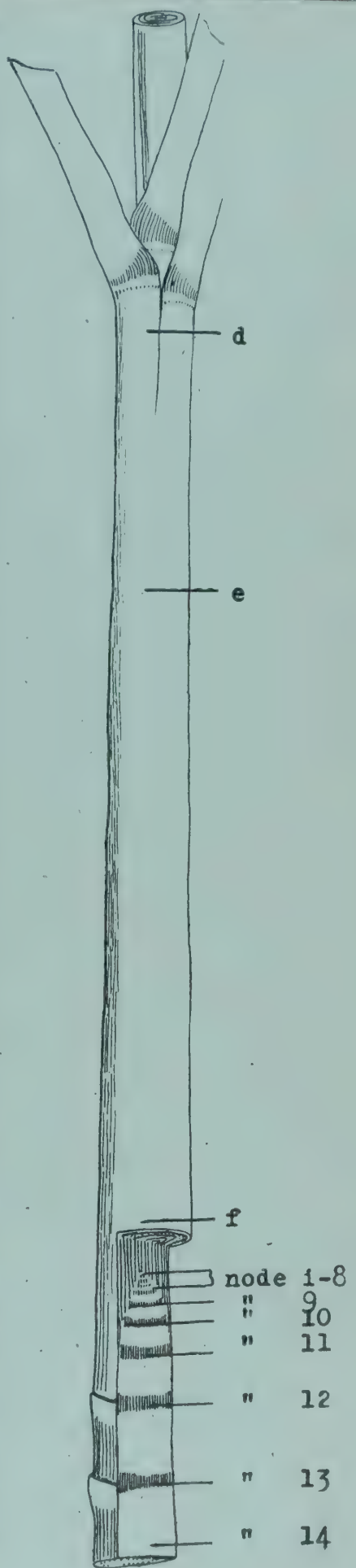


FIG. 7.—Upper part of sugar cane with leaves cut off and stem split open so as to expose the growing point and show its position in regard to the leaves. Nodes 14, 13, and 12 have leaf sheaths already mature, *d* indicates the position of the termination of the leaf sheath of node 11, *e* of node 10, and *f* of node 9. The leaf sheaths of the upper nine nodes are hardly differentiated as yet

Succession of leaves on the stem axis [a]	Length of sheath	Length of leaf blade	Succession of leaves on the stem axis [a]	Length of sheath	Length of leaf blade
	Cm.	*Cm.*		*Cm.*	*Cm.*
First	------	----	Eighth	1.2	120
Second	------	----	Ninth	3.0	155
Third	------	----	Tenth	22.0	185
Fourth	------	----	Eleventh	31.0	185
Fifth	0.1	32	Twelfth	33.0	165
Sixth	.2	50	Thirteenth	34.0	190
Seventh	.2	90	Fourteenth	33.0	186

[a] The first four leaves are not yet differentiated into sheath and blade.

Beginning with the twelfth leaf (or the youngest leaf, the blade joint of which is already superficially exposed) the length of the sheath remains practically the same and fluctuates slightly about a mean of 33 cm. While the leaf sheath is rather belated in its development the blade enlarges rapidly as soon as it is cut off from the apical meristem. Thus, a blade about 1 meter in length (at that stage still rolled in the bud) is borne by a sheath only 4 mm. long. However, although unequal at the start, blade and sheath attain their maximum length practically at the same time with the blade slightly in the lead.

The development and differentiation of the different tissues of the stem follow in sequence the external differentiation. The first eight internodes of the stem, which have a total height of a few millimeters only, show the early beginning of tissue differentiation. The growing point is made up of a thin-walled meristem, the cells of which are in a state of active division (fig. 8). Gradually certain of the cells begin to enlarge, setting off thereby small groups of cells which continue to divide actively. These groups constitute the primordia of the vascular bundles, and within them differentiation proceeds until all the components of the bundle are formed.

The protoxylem differentiates first. It is made up of a few narrow, but greatly elongated elements, which have secondary thickenings in the form of rings. The third protoxylem element is usually a spiral vessel. While differentiation of the protoxylem proceeds in centrifugal direction, there appear on the opposite pole of the group the first elements of the phloem recognizable by the refractiveness of their thickened walls. Between xylem and phloem there is an actively dividing tissue which sometimes takes on the appearance of a typical cambium and from which all other xylem and phloem cells are formed.

Not all bundles in a cross section of the growing point show the same degree of differentiation. A certain number of them are larger and contain a greater number of protoxylem cells. The larger xylem elements have closely ringed wall sculpture and comparatively short articulations. In a cross section just below the insertion of the young leaf these bundles are seen to bend more or less abruptly and move toward the periphery, where they enter the leaf. They constitute the large leaf traces which already in their very young condition foreshadow the structure characteristic of the mature traces. The peripheral stem bundles are much smaller than those nearer the center with many of them just in the state of procambial differentiation.

Cross sections through the tenth internode (just 1 cm. below the growing point) show the differentiation of the large pitted vessels. The walls of these cells are still very thin and easily crushed in making a section. The tissue surrounding the xylem and phloem is composed of narrow, elongated parenchyma cells which gradually mature into the bundle sheath. The development of the bundle sheath, however, is very slow and is completed only after all the vascular elements of the bundle are fully formed.

In the twelfth internode the large vessels have developed a secondary wall which shows the pitting characteristic of the mature element. The cross walls between the articulations of the vessels have also disappeared and communication is now established through large pores. As the large vessels complete their development the phloem cells divide longitudinally to form the sieve tubes and the companion cells of the mature bundle.

In the next lower internode the walls of the large vessels have become lignified. The cells of the sheath have ceased division and have begun to thicken their walls, which is especially pronounced in the cells forming the bundle caps.

Finally, in the fourteenth internode, with the lignification of the sclerenchyma sheath, the formation of the protoxylem lacuna and the obliteration of the protophloem, the stem bundle is completely mature. The formation of the protoxylem lacuna may be observed at an earlier stage, but then only in individual bundles.

Hand in hand with the progressive differentiation of the different elements of the vascular bundle goes an increase in the size of the cells. This increase, however, is not so marked in the bundle

itself as in the surrounding parenchyma. The cells of this tissue, at a time when the large vessels are just differentiating, are about 65μ in width. When the bundles are fully developed, the parenchyma cells measure 125μ and may attain even greater dimensions. This increase in size of the parenchyma cells is in a large measure responsible for the enlargement of the stem, since the maximum number of bundles present in a stem is reached long before the latter has attained its final thickness.

The differentiation of the tissues of the leaf sheath has been described by Kobus (*10*). It takes place in the same sequence as in the stem, only it seems to be more rapid. Thus an internode which bears a leaf sheath 3 to 4 cm. long has only a few mature protoxylem elements, while in the leaf sheath the large vessels are already fully developed, although not as yet lignified.

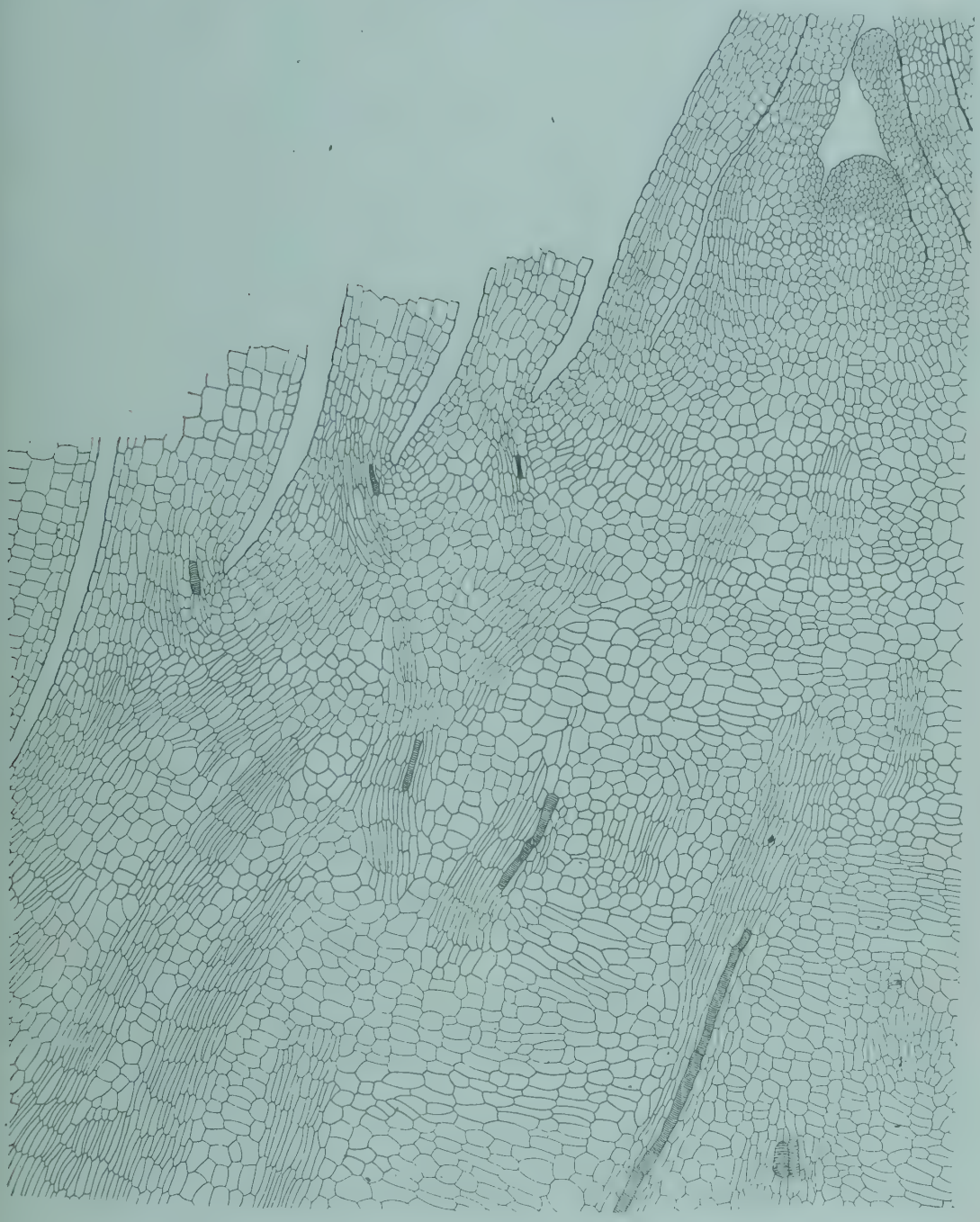


FIG. 8.—Longitudinal section through the growing tip of a large sugar cane. × 225

The phloem of the leaf sheath also develops in advance of that in the stem, since differentiation into sieve tubes and companion cells has already taken place.

The epidermis of the young stem and of the lateral organs is made up of thin-walled rectangular cells (pl. 11, B).

In their further development these cells either merely enlarge as, for example, the cells of the inner epidermis of the leaf sheath and the ligule, or they undergo further modifications. In the latter case each cell divides unequally giving rise to a long cell and a short cell. The long cell elongates still further, while its walls become thick and strongly undulated. The short cell either ceases growth and undergoes silicification, or, according to Pfitzer (*14*), it becomes the primordium of a hair or stomate.

In the development of a hair the peripheral tangential walls of the epidermal cell begin to show marked apical growth. As the cell enlarges, the nucleus divides (pl. 14, A). The binucleate condition exists until the cell has attained approximately its full length; a wall then develops which divides the hair into two more or less equal parts. In the later development, the base of the hair becomes lignified, while in the apical region there is often found a slight brownish precipitate. The first-formed hairs are commonly two-celled. They are always delicate and ephemeral. Short unicellular hairs with thick, lignified walls are characteristic of the more mature organs. In the region of the sheath and blade-joints the unicellular hairs attain a considerable length, which reaches its maximum in the fringes at the base of the ligule.

The short cells of the epidermis next to the veins of the leaf and more sparingly in other regions continue development into stomates. The short cell constitutes the stomate mother cell which by further division gives rise to the two guard cells. The two subsidiary cells of the stomate are formed as lateral outgrowths from the adjacent parenchyma cells. With the formation of the stomatal chamber and the separation of the wall between the two guard cells the development of the stomate is complete. The stomates of the inner epidermis of the leaf sheath differ from those of the stem and leaf blade in that the subsidiary cells are very broad; the guard cells too appear to be slightly different and the stomatal pore instead of being a narrow cleft of even width is more or less oval (pl. 19).

The epidermal cells of the stem remain thin-walled for a long time, even after the vascular bundles are fully developed. Eventually, however, the walls thicken, especially the outer ones, which in addition become cuticularized and finally covered with a layer of wax. The epidermis of the leaf blade and the outer epidermis of the sheath also become thick-walled and lignified.

SUMMARY

The vascular bundles of the sugar cane are of the normal monocot type, rhomboid to oval in cross section and surrounded by a well-marked sclerenchymatous sheath which is enlarged on the phloem and xylem poles into bundle caps. Phloem and xylem are disposed collaterally in relation to each other. Deviations from this arrangement are observed in the node and the lower leaf sheath, since here certain of the bundles show a more or less amphivasal structure. True amphivasal arrangement, however, is found only in the larger traces of the buds. In the lower leaf sheath a pseudo-amphivasal arrangement is sometimes produced by the formation of compound bundles.

The xylem is composed of some protoxylem and two large pitted vessels. The protoxylem forms a short radial row of annular and spiral elements. It is more extensive in the node than in the internode and reaches its maximum development in that part of the leaf traces which runs horizontally in the node. The large pitted vessels of the metaxylem also tend to increase in number as the vascular bundle passes through the node; the large horizontal leaf traces on the other hand lack the large vessels altogether. The internodal bundles, except those near the periphery, and the large bundles of the leaf sheath and the blade possess a more or less conspicuous protoxylem lacuna. The lacuna is absent in the bundles of the node, the peripheral bundles of the internode, and the smaller bundles of the leaf.

The phloem consists of sieve tubes and companion cells. It is most extensive in the bundles of the node and the leaf sheath, but least conspicuous in the smaller bundles of the leaf blade and the peripheral bundles of the internode. Changes in the phloem consist in obliteration of the protophloem, which is most pronounced in the large bundles of the leaf, probably because of the development of an inner fibrous sheath above the phloem cells and in a lignification of certain portions of the mature phloem tissue. Lignified metaphloem is most frequently observed in the large bundles of the node and in the Keimring.

The vascular bundles are surrounded by a sclerenchymatous sheath which is most strongy developed on the inside

and the outside of the bundle, where it forms typical caps. In the peripheral internodal bundles the sheath increases greatly but mostly on the xylem pole, whereas in the nodal bundles the sclerenchyma caps on the phloem pole are most conspicuous. In the bundles of the leaf blade the cells of the sclerenchyma sheath become larger, more regular, and contain large chloroplasts. In addition, the leaf bundles possess a fibrous sheath located between the chlorophyll-bearing sheath and the phloem. The extent of this fibrous sheath is in direct relation to the size of the bundle.

The epidermis of the stem and of the lateral organs is made up of two types of cells—long cells with strongly undulated walls and short silicified cells. The inner epidermis of the leaf sheath and the ligule, however, consists only of long cells. The epidermis of the mature stem is strongly lignified and covered with a layer of wax. The subepidermal cells also become lignified, but thin-walled parenchyma cells are interpolated at intervals. The epidermis of the leaf sheath and the blade bears hairs and stomates of a type common to grasses.

The vascular tissue of the root forms a siphono stele bounded externally by a cortex, internally by a pith. The peripheral cortical cells are suberized, forming a well-marked exodermis. Between the pith and the vascular cylinder is a sclerenchymatous layer which becomes lignified very early, and affords support to the young and delicate vascular tissue of the root. The xylem plates of the root are separated from the endodermis by a single-layered pericycle, though in small, lateral rootlets a xylem cell may border directly on the endodermis.

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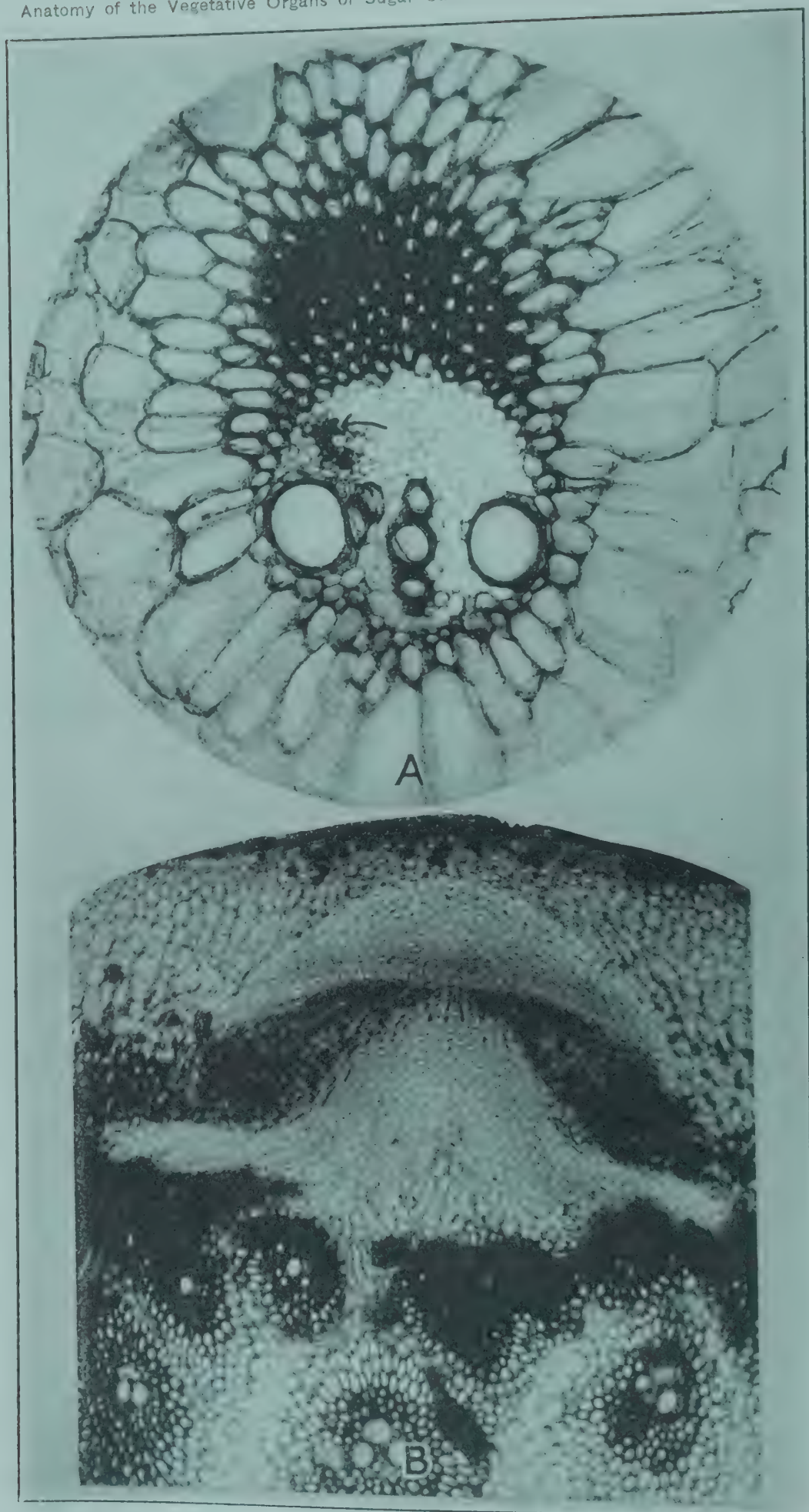
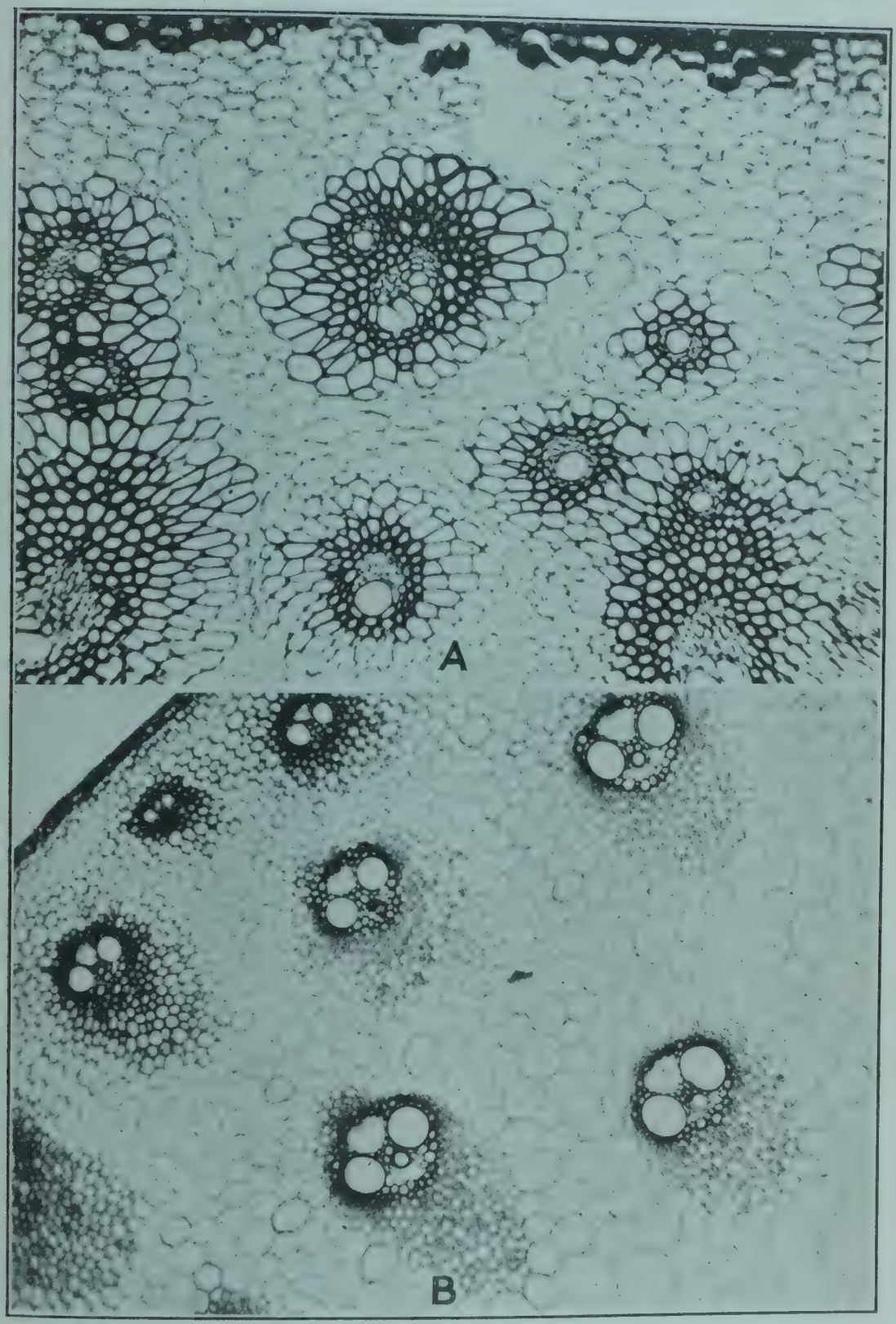
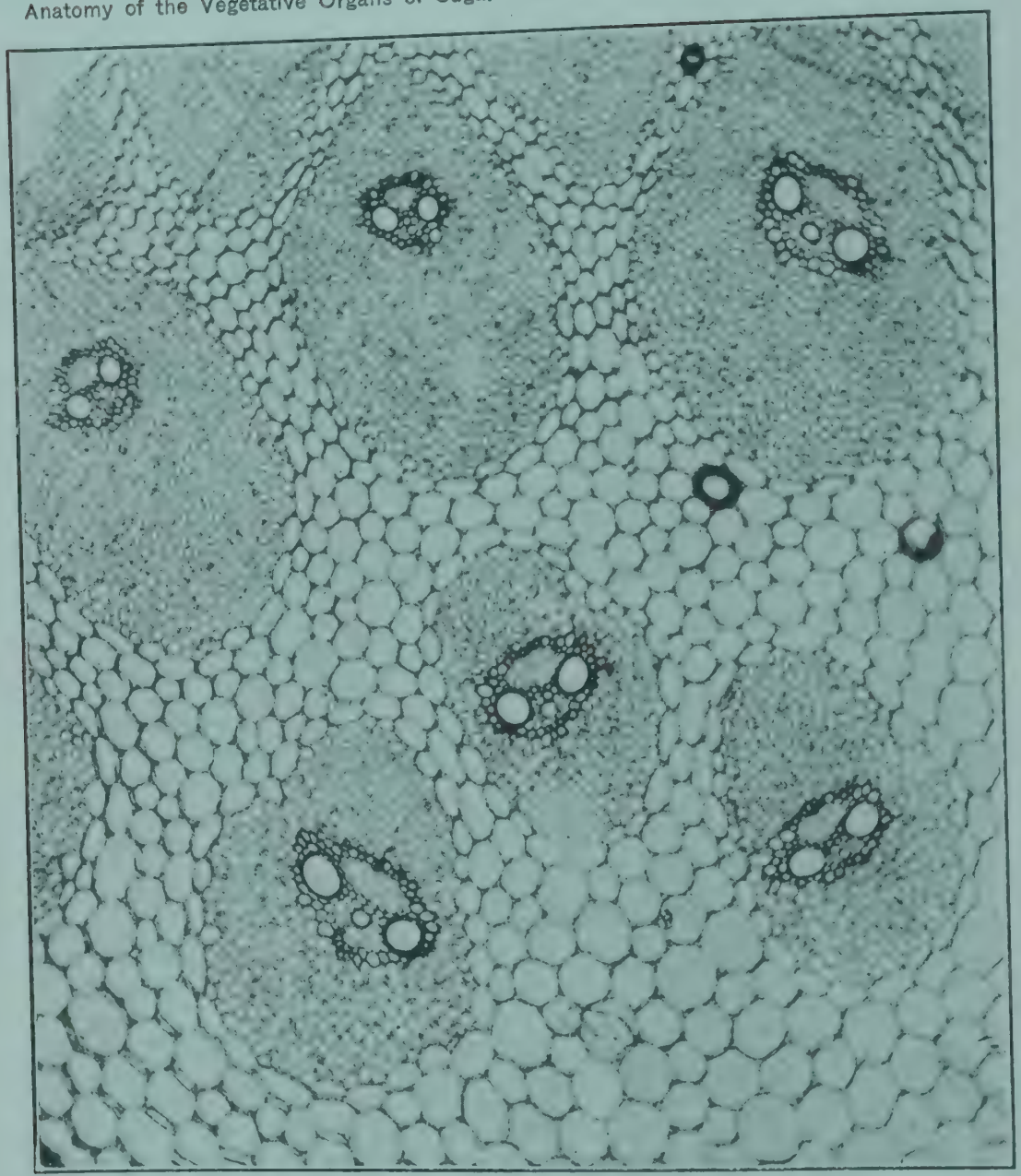


A.—Cross section of mature nodal bundle. Notice the lignification in the phloem (designated by the arrow). × 124

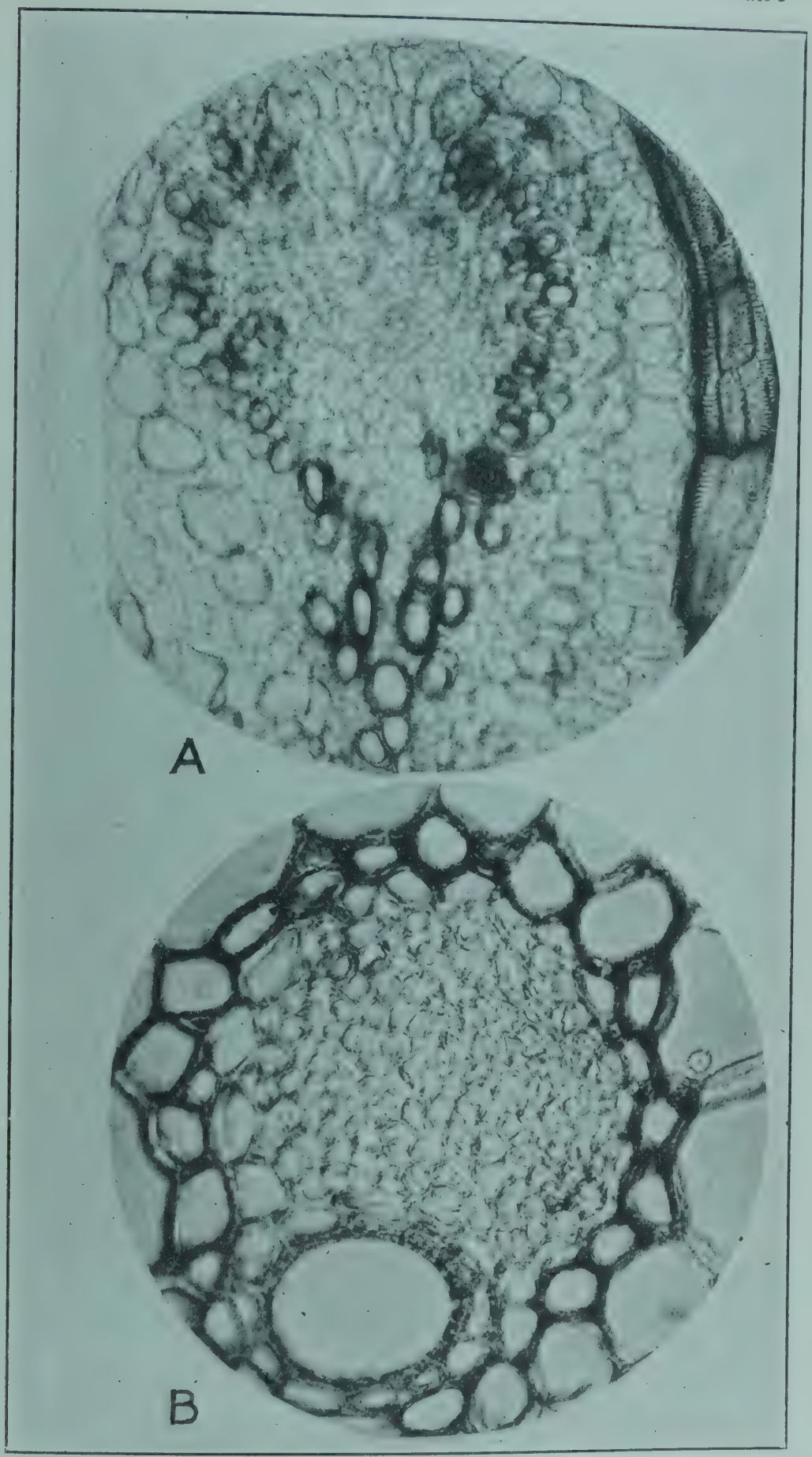
B.—Cross section through the peripheral part of the Keimring showing a root primordium. × 80



A.—Cross section through outer region of node of mature stem. × 106
B.—Cross section through peripheral part of internode of mature stem. × 85



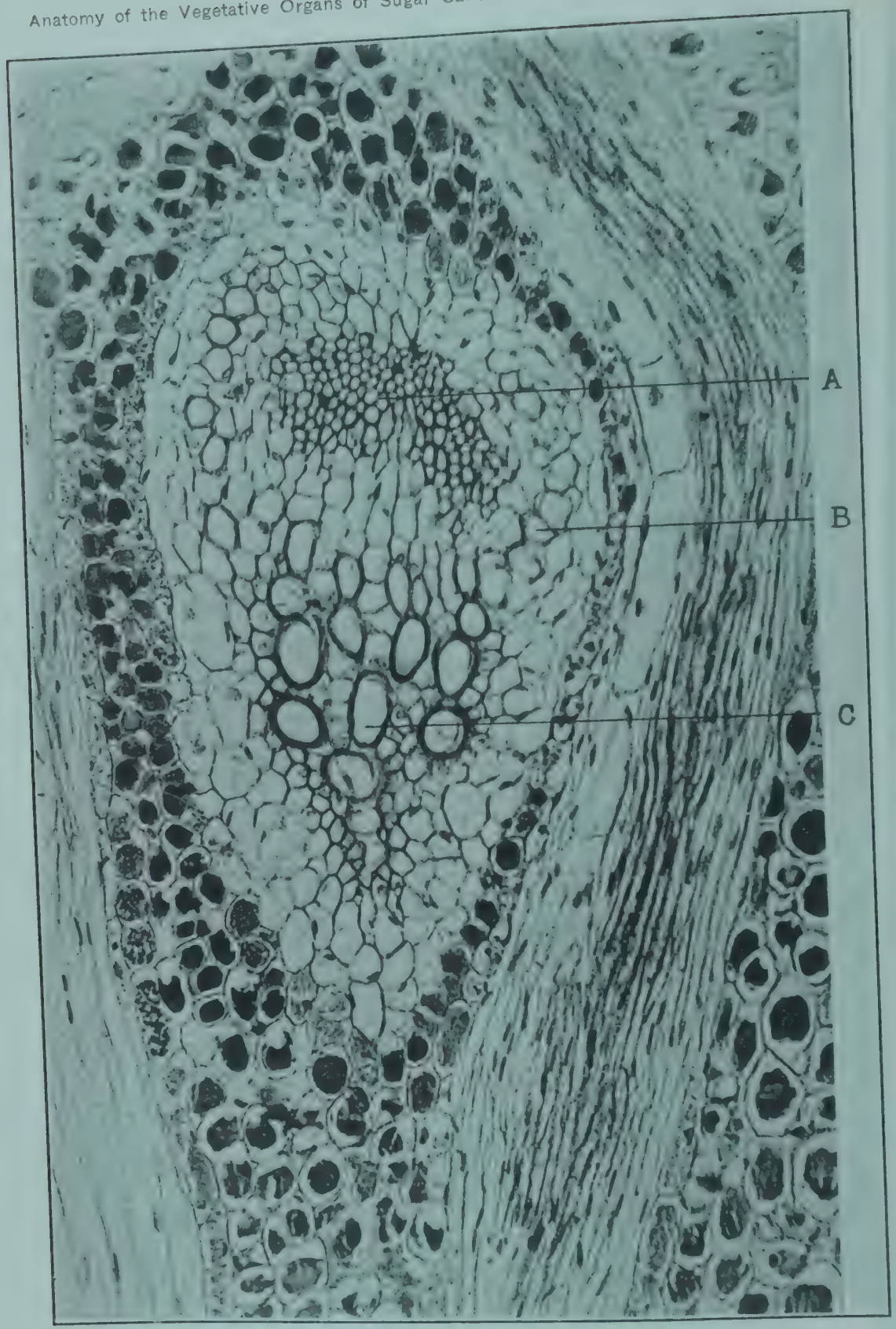
Cross section through the peripheral region of the intercalary meristem of internode (hand section of fresh material). × 188



A.—Leaf trace cross section as it appears in its longitudinal course through the node prior to passing out into a leaf. × 200

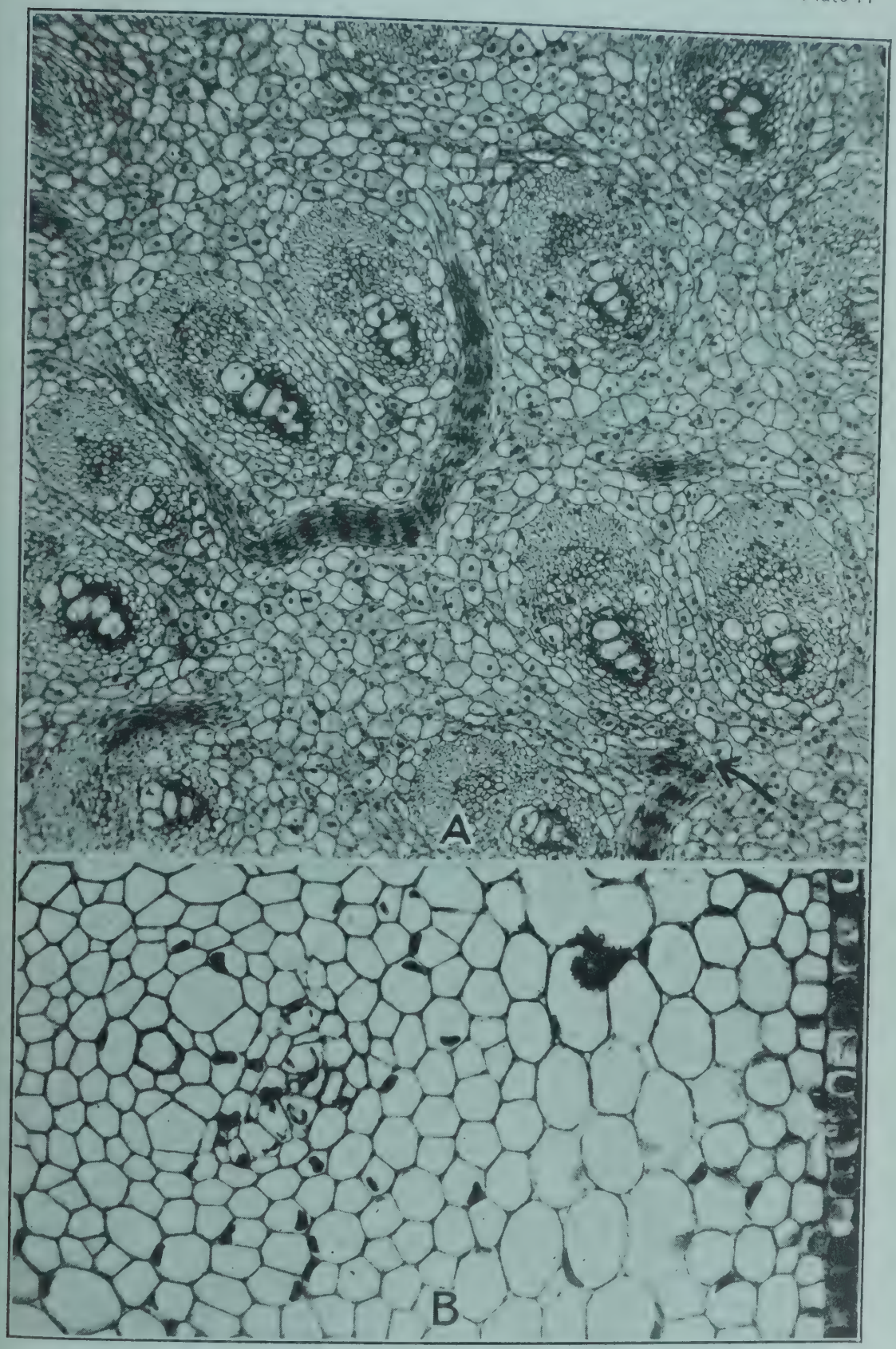
B.—Cross section of one of the numerous horizontal bundles of the node. There is only one xylem element, but a great deal of phloem. × 510

Anatomy of the Vegetative Organs of Sugar Cane



Cross section of l arge leaf trace as it appears in its longitudinal course through a node near the apex of the stem. There is a great deal of protoxylem and a few scattered scalariform elements at the flanges of the bundle. The bundle is surrounded by parenchyma cells which have a dark granular deposit of tannin and other substances. × 198

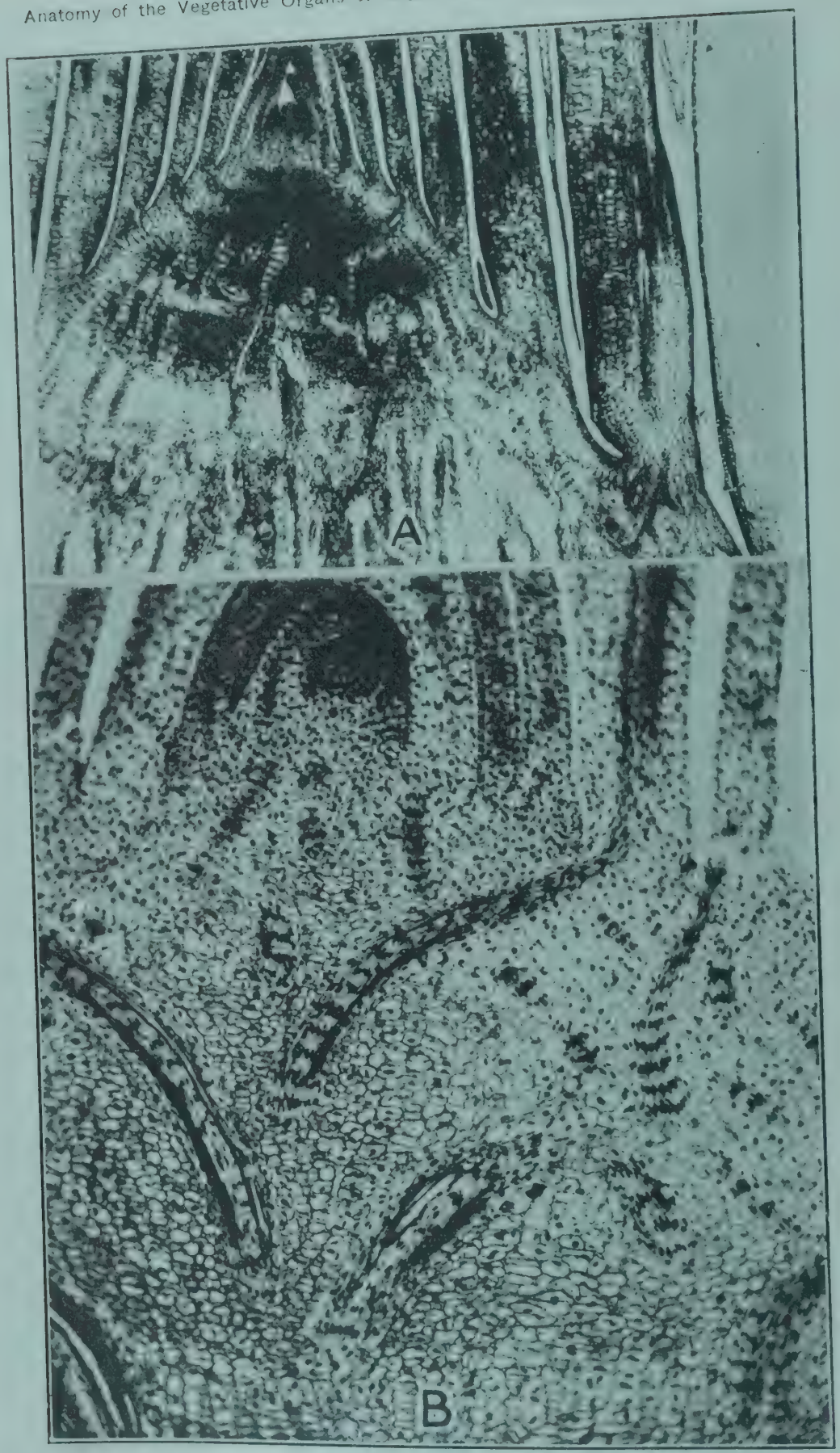
A.—Phloem
B.—Scalariform xylem elements
C.—Protoxylem



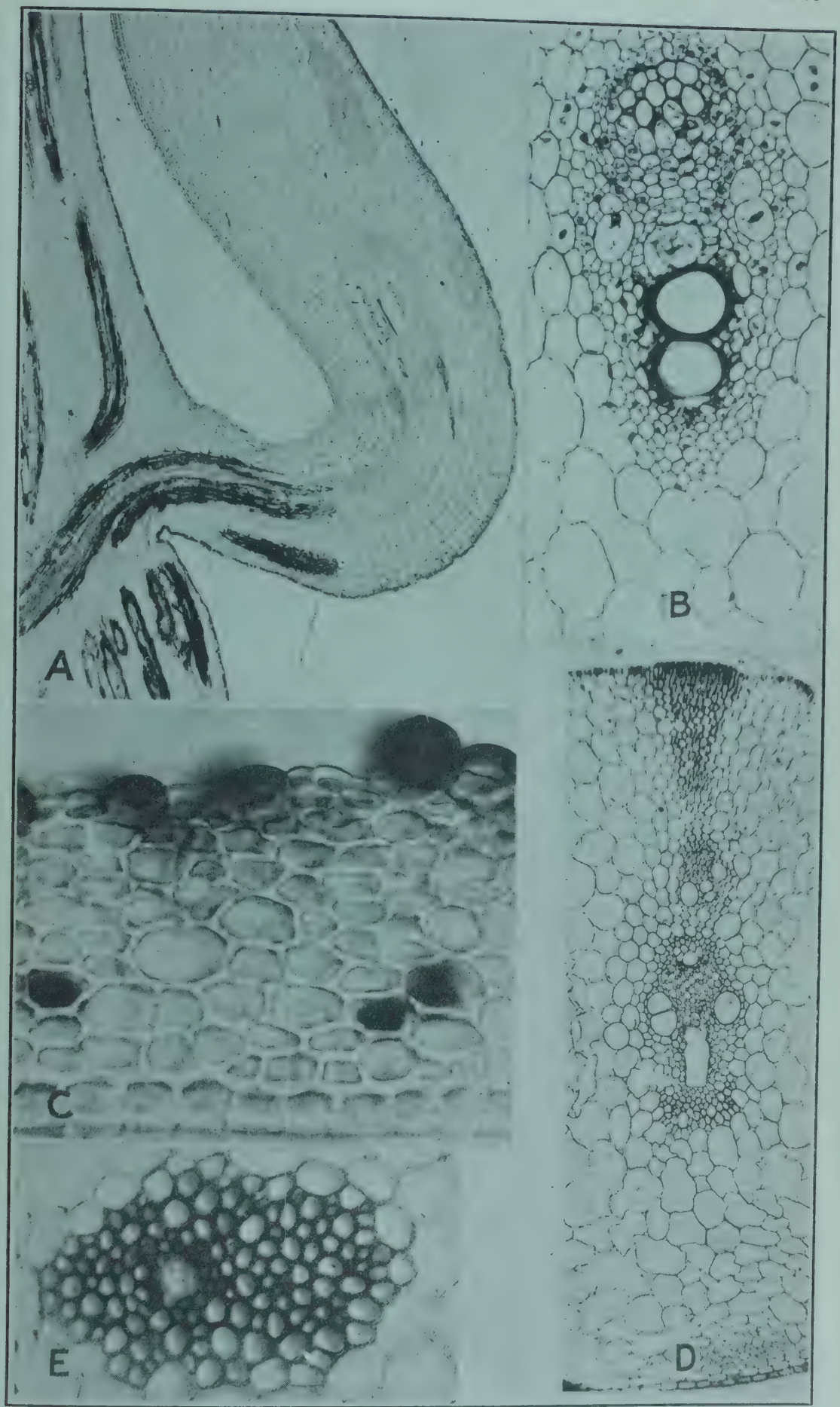
A.—Cross section through the central part of a young node. The vascular bundles are elongated radially. There is a great deal of protoxylem. The section shows also some of the smaller bundles cut longitudinally (designated by the arrow). × 76

B —Section through a young internode showing epidermis, cortex, and vascular bundles in the state of differentiation. × 468

Anatomy of the Vegetative Organs of Sugar Cane

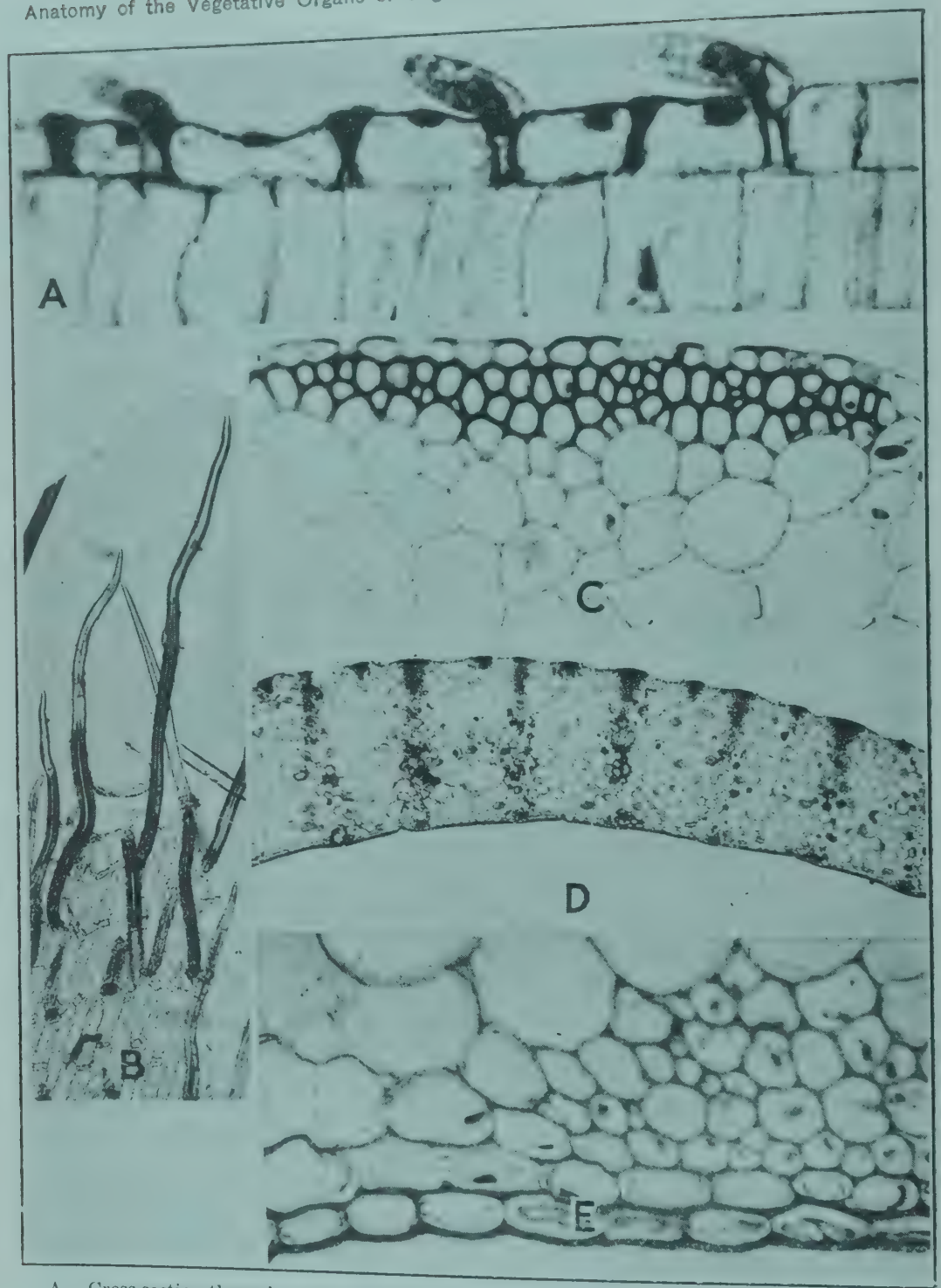


A.—Longitudinal section through the growing point of a large stem. × 35
B.—Longitudinal section through the growing point showing the curved path of the leaf traces. Notice the crossing of the traces. × 83

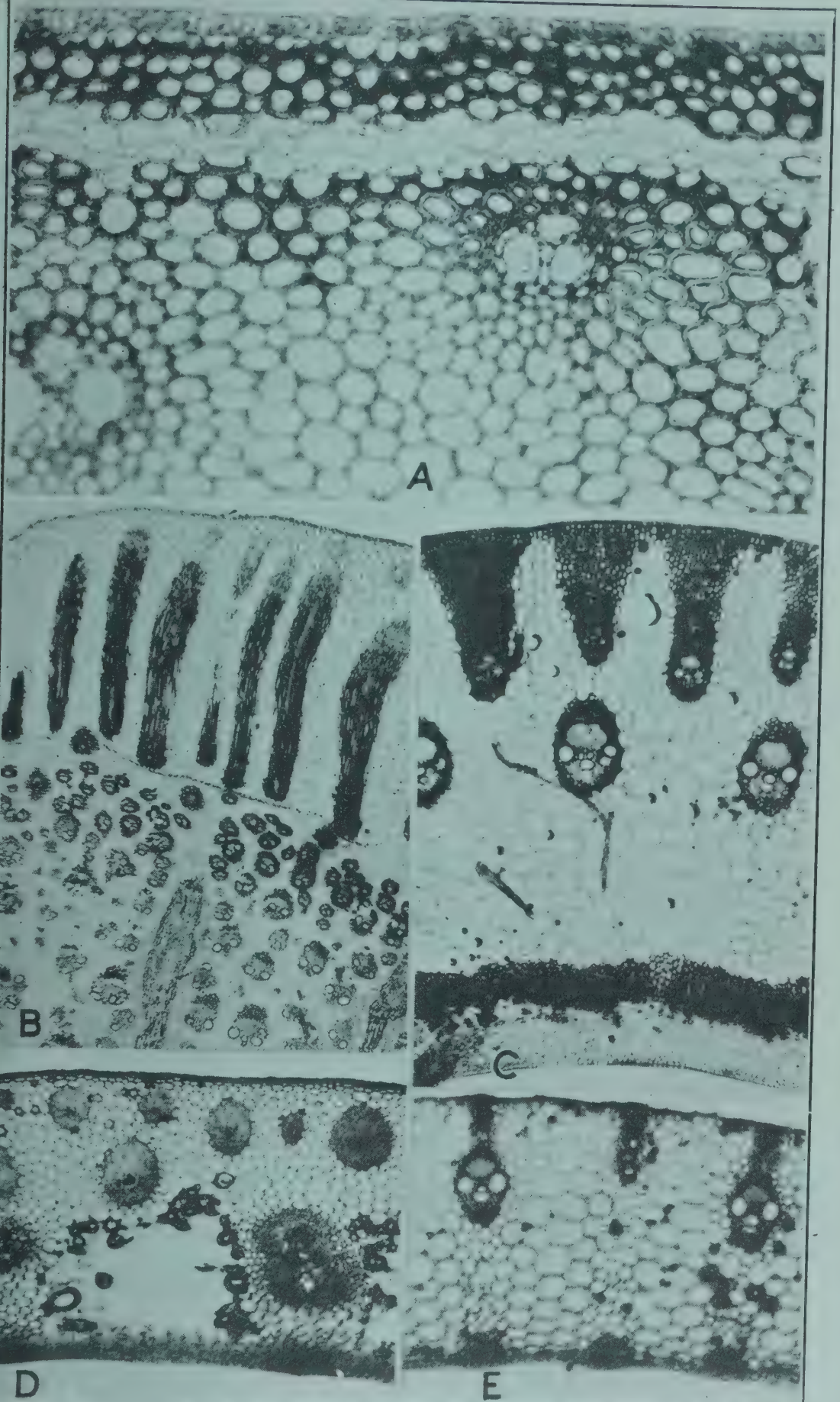


A —Longitudinal section of sheath joint to show the passing out of a large leaf trace. × 19
B —Cross section through a young bundle of leaf sheath. Between xylem and phloem is a cambium-like tissue. × 188
C —Cross section through ligule (hand section of fresh material). The dark circles above are hairs cut across. × 480
D —Cross section through a young leaf sheath. For detail see Figure 4. × 66
E —Cross section through a collenchyma bundle inclosing a few phloem cells. × 188

Anatomy of the Vegetative Organs of Sugar Cane



A.—Cross section through a young leaf showing development of hairs. × 468
B.—Epidermis and hairs from sheath joint. × 104
C.—Cross section through young leaf sheath. The epidermis shows both long and short cells; the latter are silicified and appear white in the picture. Beneath the epidermis is a group of lignified sclerenchyma cells. × 412
D.—Cross section through young leaf sheath showing general arrangement of the tissues. × 16
E.—Enlarged view of lower epidermis of the leaf sheath. Notice that the epidermal cells are larger than those shown in C, that the cells of the sclerenchyma are also larger, not lignified and separated from the epidermis by a layer of parenchyma cells. × 321



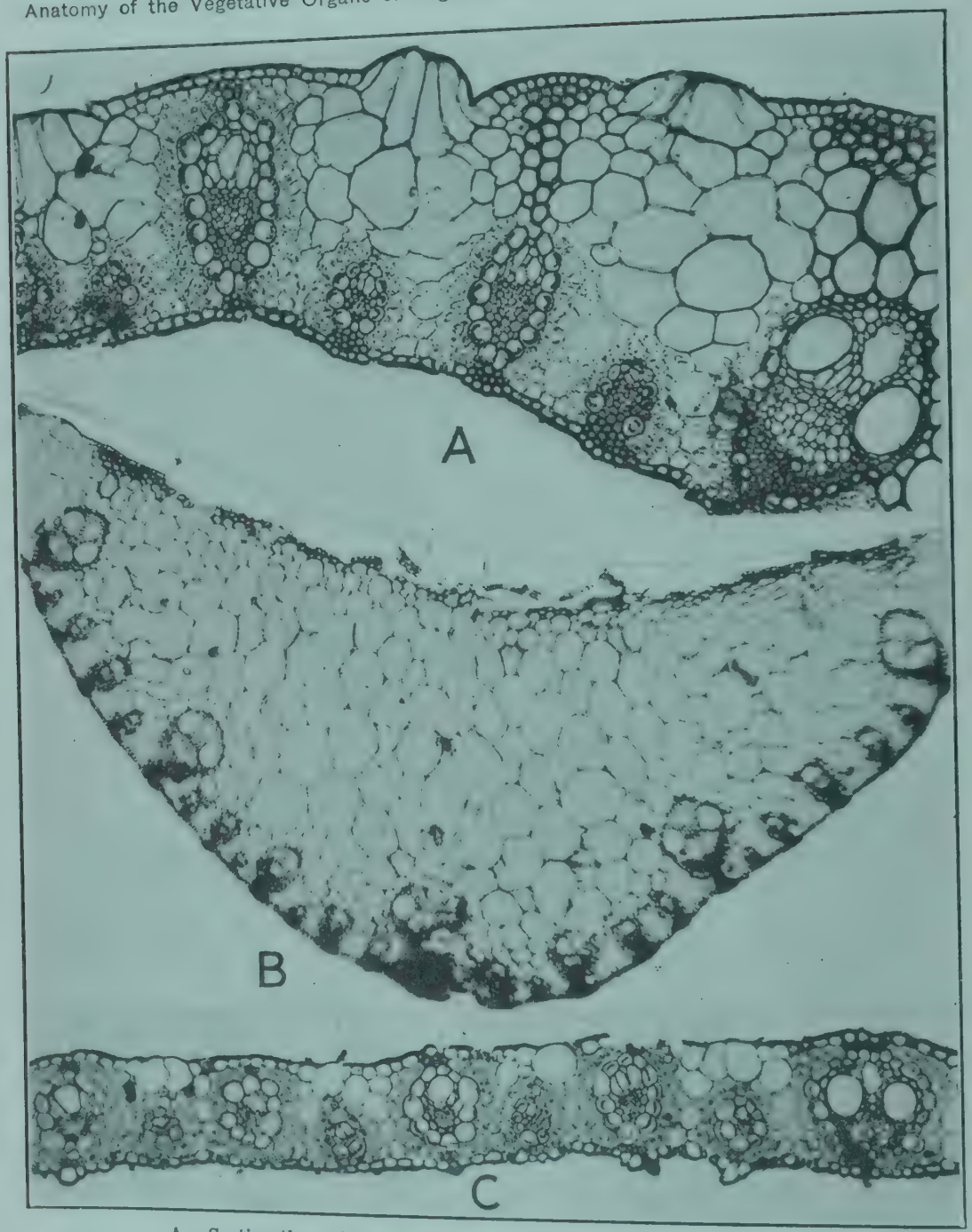
A.—Cross section through peripheral region of mature internode. Beneath the epidermis are several layers of lignified cells, and beneath these are two or three layers of thin-walled parenchyma. For a longitudinal section through this region see text figure 2. ×188.

B.—Cross section through outer stem and leafsheath at the point of the insertion of the latter on the stem. ×19.

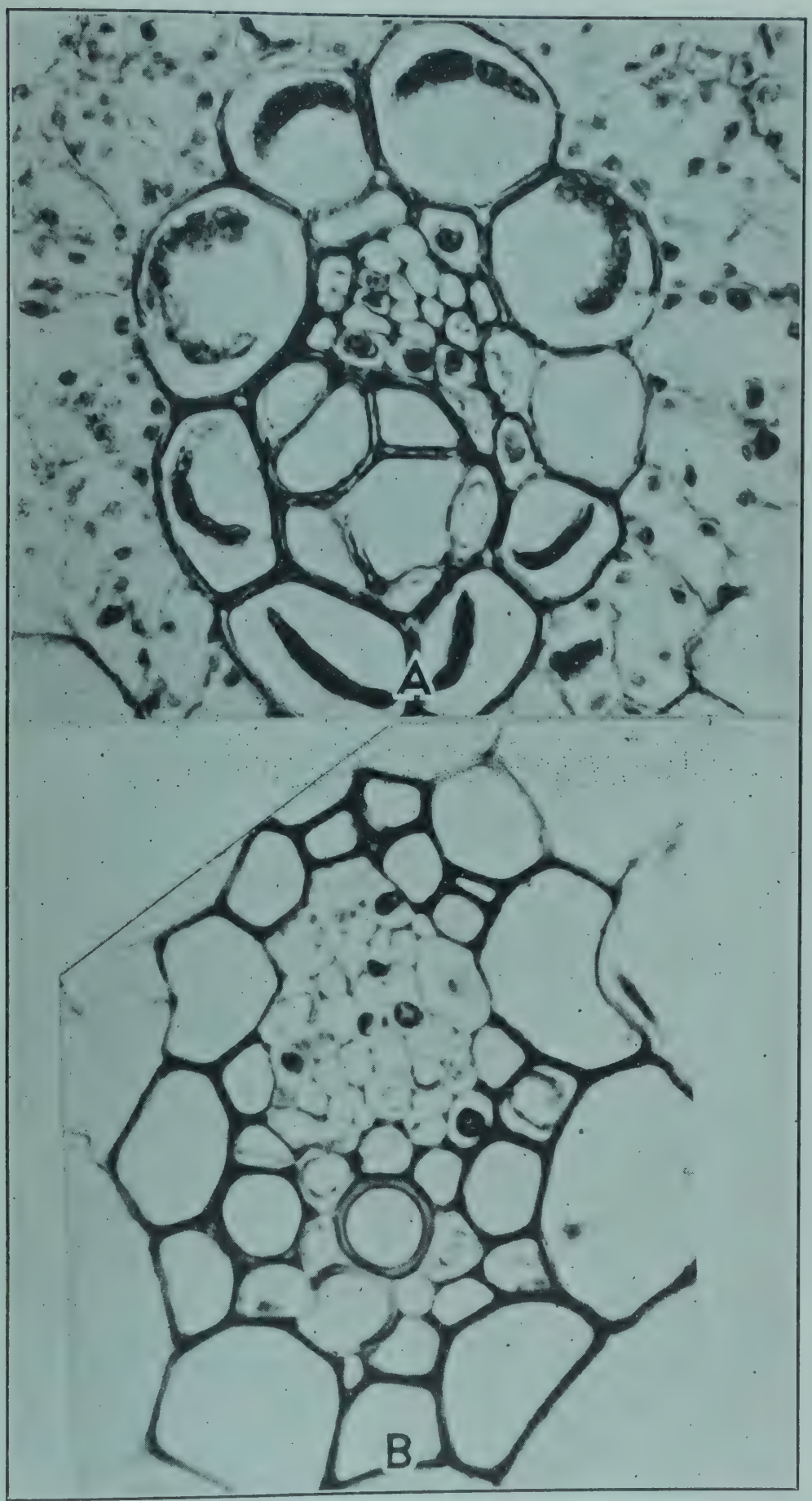
C.—Cross section of the sheath at the insertion point of the ligule. The section appears broad since it is taken through the central part of the sheath and not the flanges. ×24.

D.—Cross section through the sheath-joint. ×24.

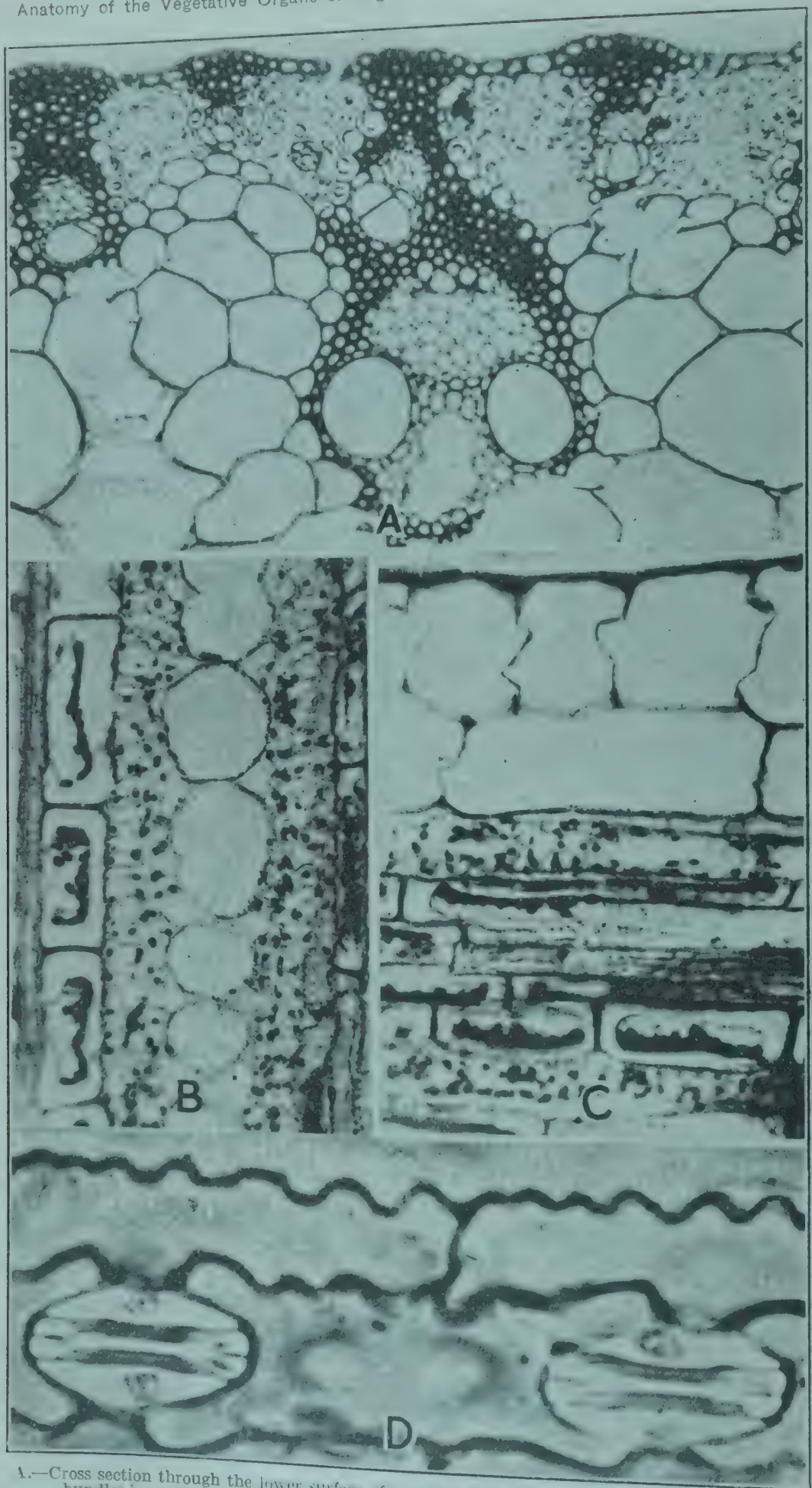
E.—Cross section through leafsheath midway between the sheath-joint and the blade joint. ×24.



A.—Section through leaf blade in the region near the midrib. × 106
B.—Section through the midrib. × 34
C.—Section through a mature leaf blade. × 88



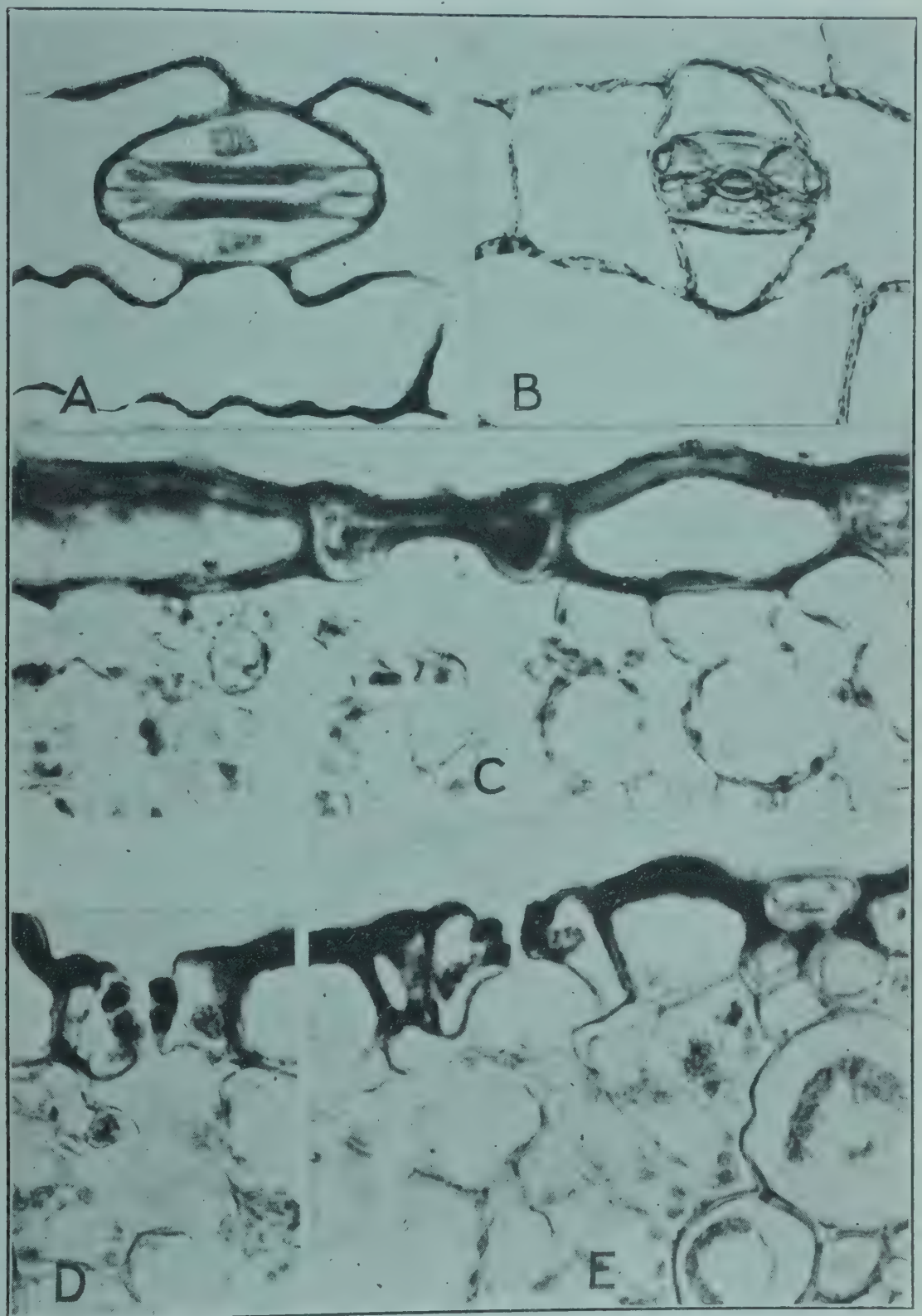
A.—Cross section through a small vascular group of a leaf. × 890
B.—Cross section through a small vascular group of the leaf sheath. The large-celled sheath around the bundle corresponds to the sheath in Figure A, but the chloroplasts are wanting. The phloem is well developed, the xylem consists of few spiral elements; a protoxylem lacuna is wanting. × 970



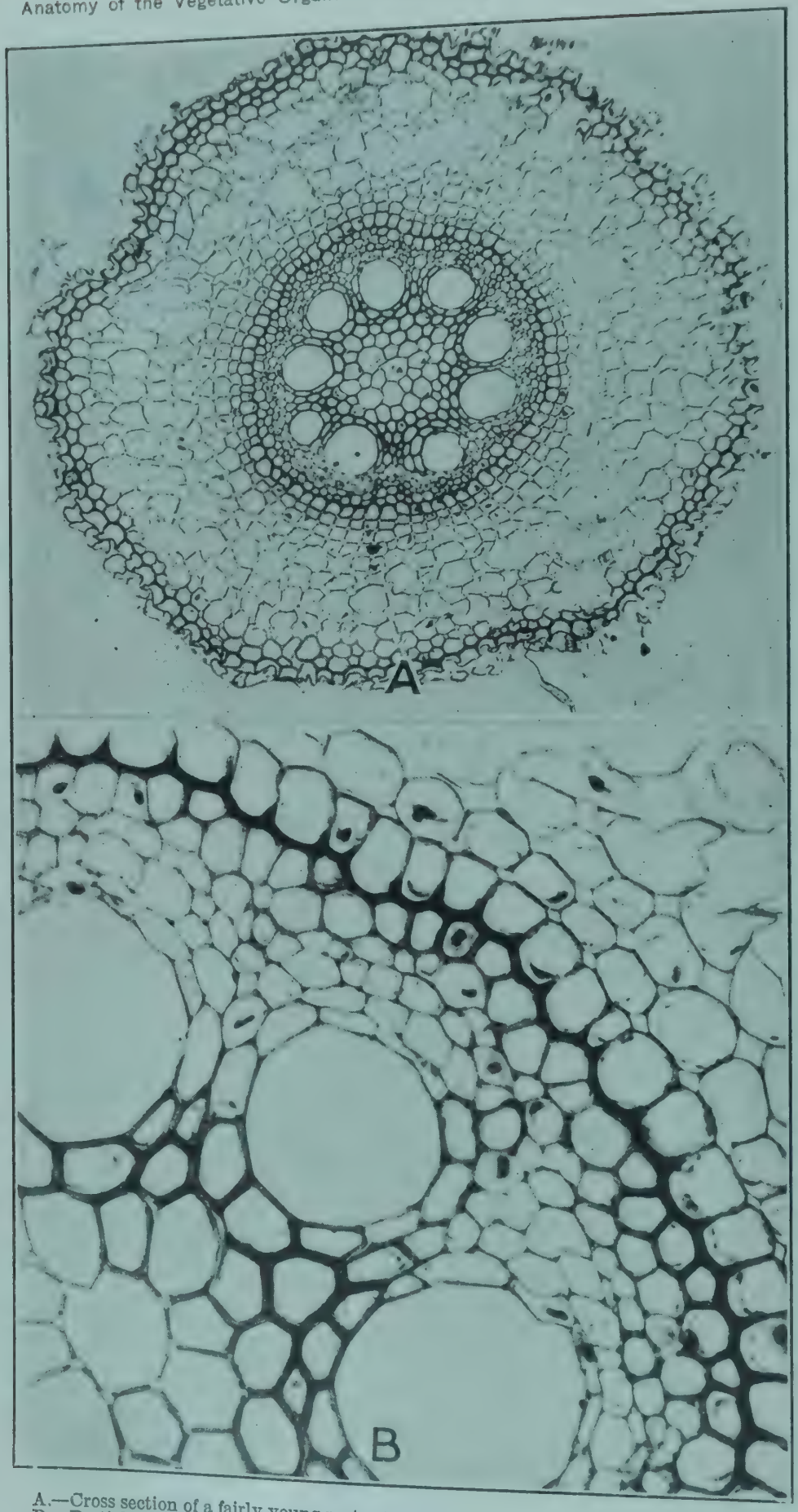
A.—Cross section through the lower surface of mature midrib. The sclerenchyma of the large bundles is continuous with the lignified epidermis. ×165.

B.—Tangential section through a mature leaf. From left to right: Sclerenchyma fiber, chlorophyll-bearing bundle sheath, spongy parenchyma, bulliform cells, spongy parenchyma. ×340

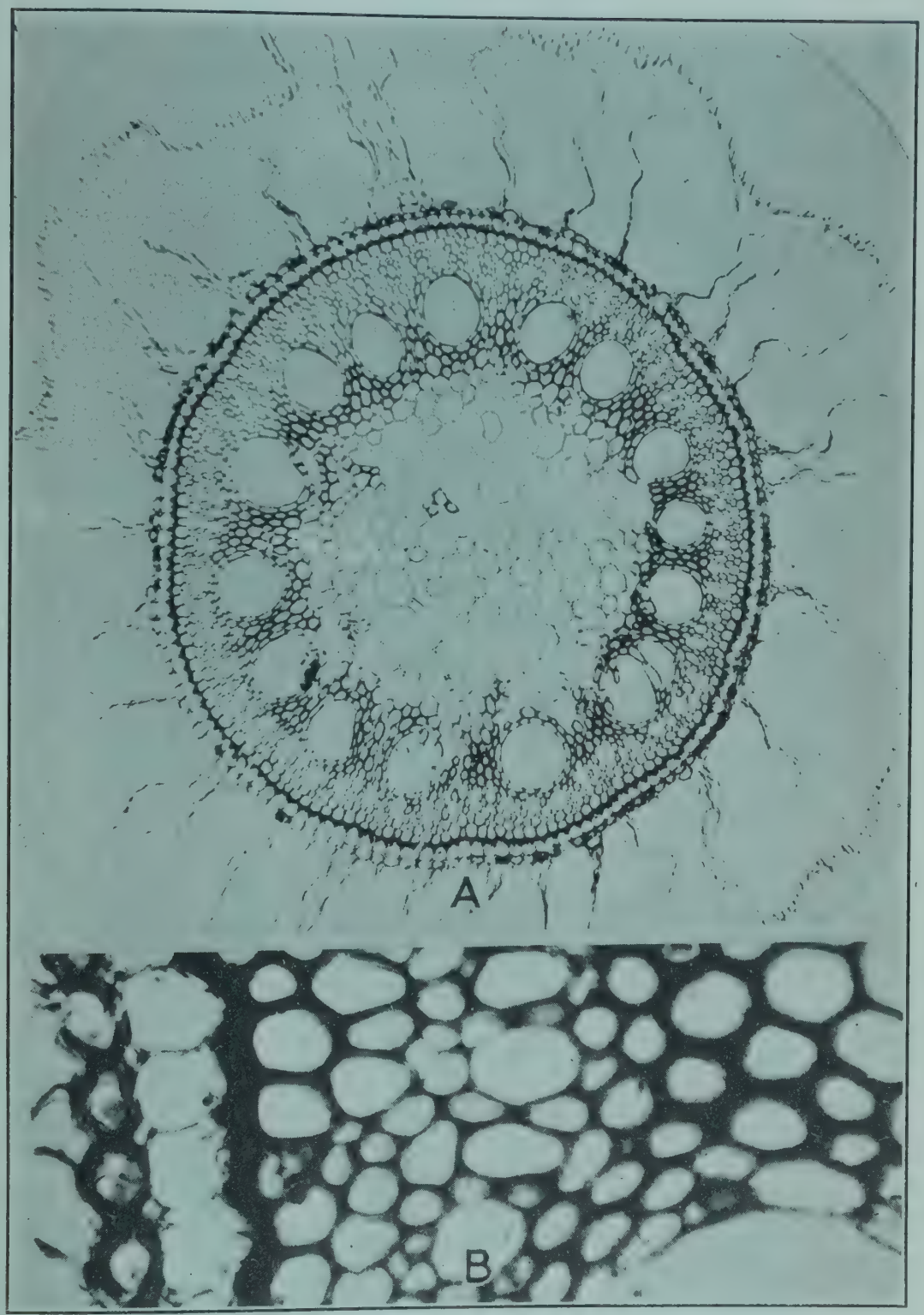
C.—Radial section through a mature leaf. The upper epidermis in this section [illegible] bulliform cells ×340.



A.—Surface view of stomate of a leaf. × 840
B.—Surface view of stomate of inner epidermis of leaf sheath. × 480
C.—Longitudinal section through a stomate of a leaf. × 840
D.—Cross section through a stomate of a leaf. × 840
E.—Cross section through a stomate of a leaf The second epidermal cell to the right is a short, silicified cell. × 840

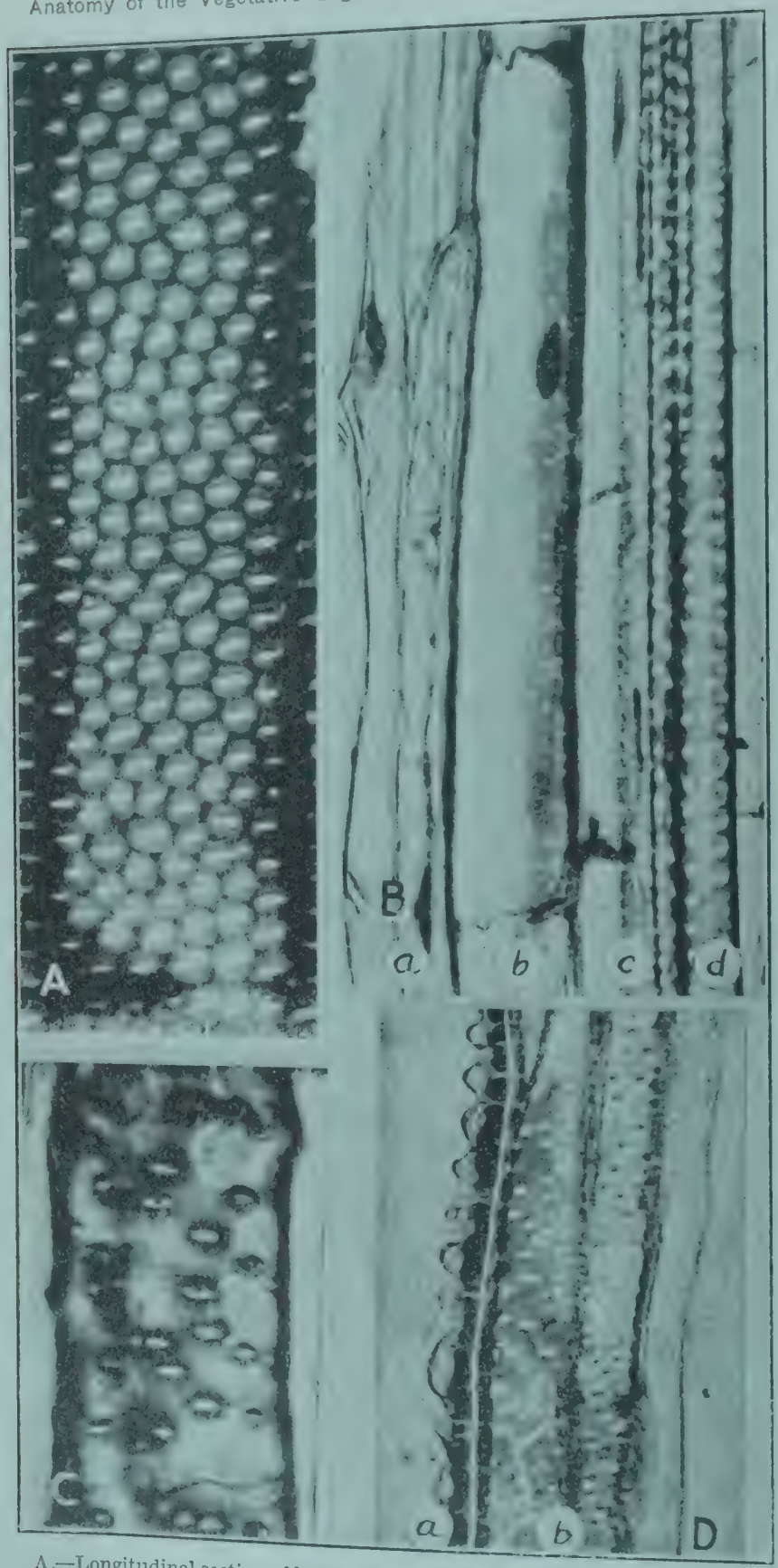


A.—Cross section of a fairly young root. × 113
B.—Partial enlargement of Figure A, to show details in structure of endodermis and vascular tissue. × 510

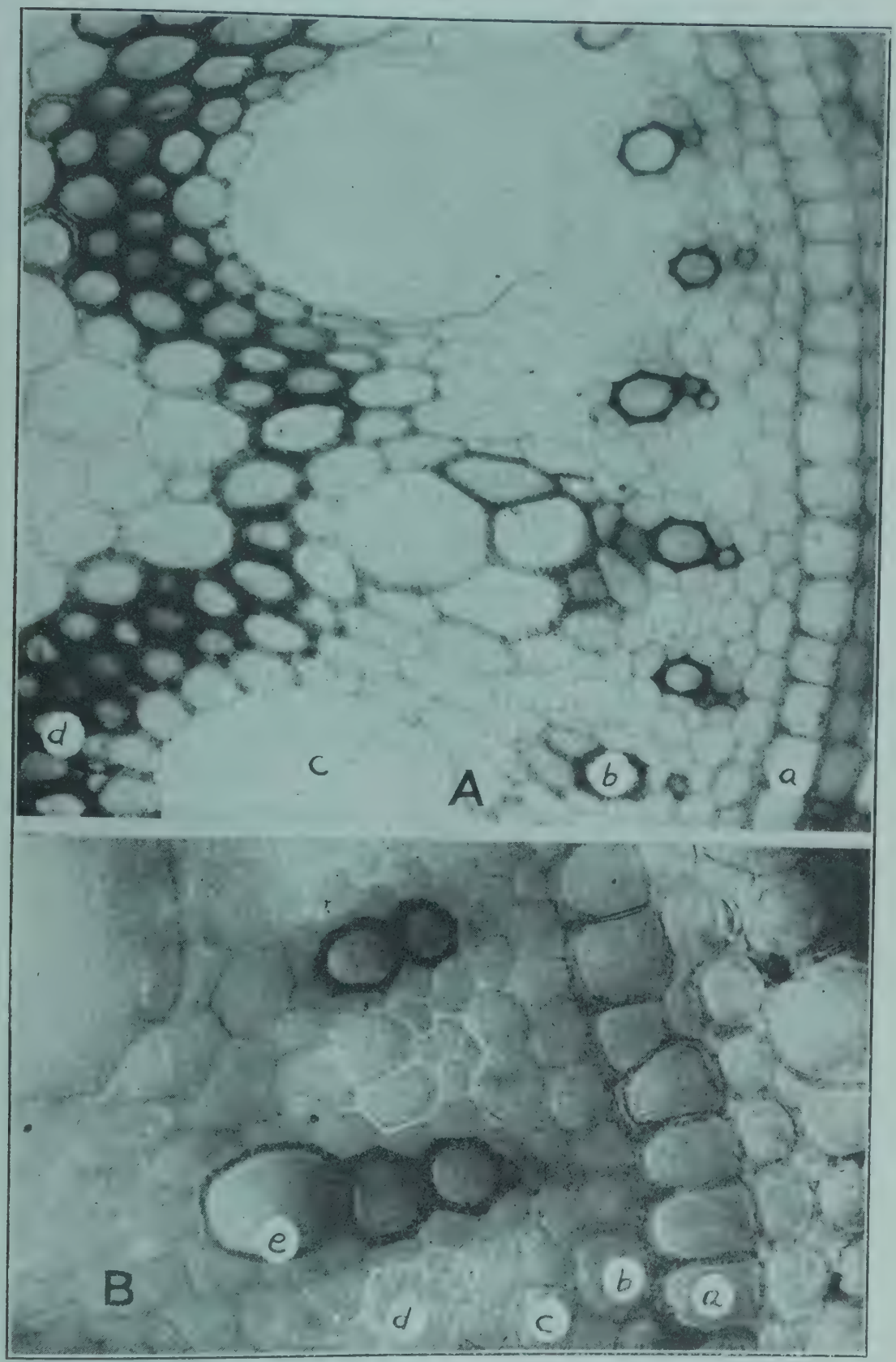


A.—Cross section of a large root. × 76
B.—Enlarged view of A. The row of cells at the extreme left has thickened and suberized walls. Lumen is often filled with gum. × 432

13951—25†——4

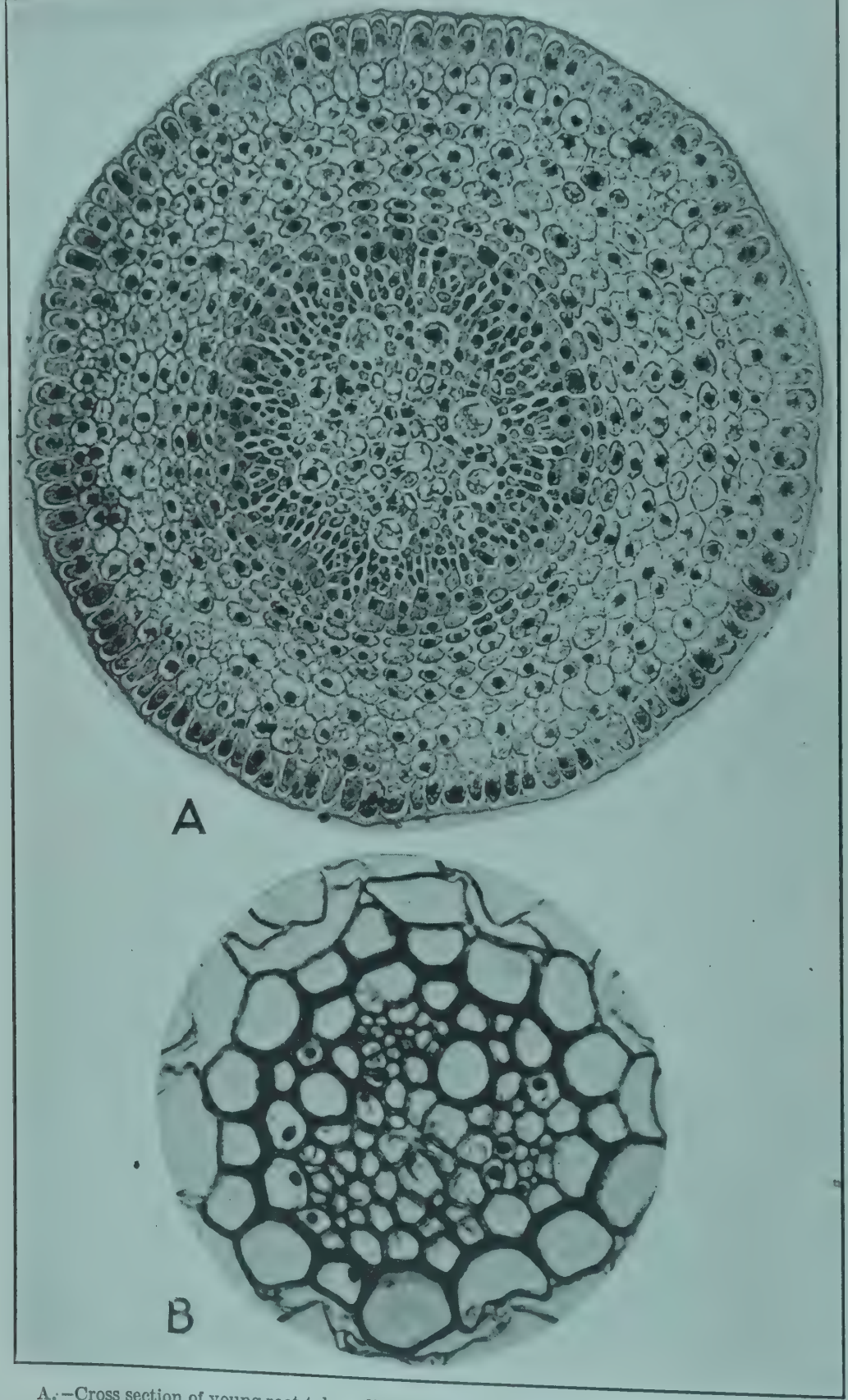


A.—Longitudinal section of large pitted vessel of root. × 1040
B.—Longitudinal section through vascular tissue of root. × 510. a, Inner cortex; b, endodermis; c, pericycle; d, protoxylem
C.—Longitudinal section of endodermis. × 1040
D.—Longitudinal section through vascular tissue of root. × 510. a, Endodermis showing the wartlike protuberances of the tangential wall and the pits between them

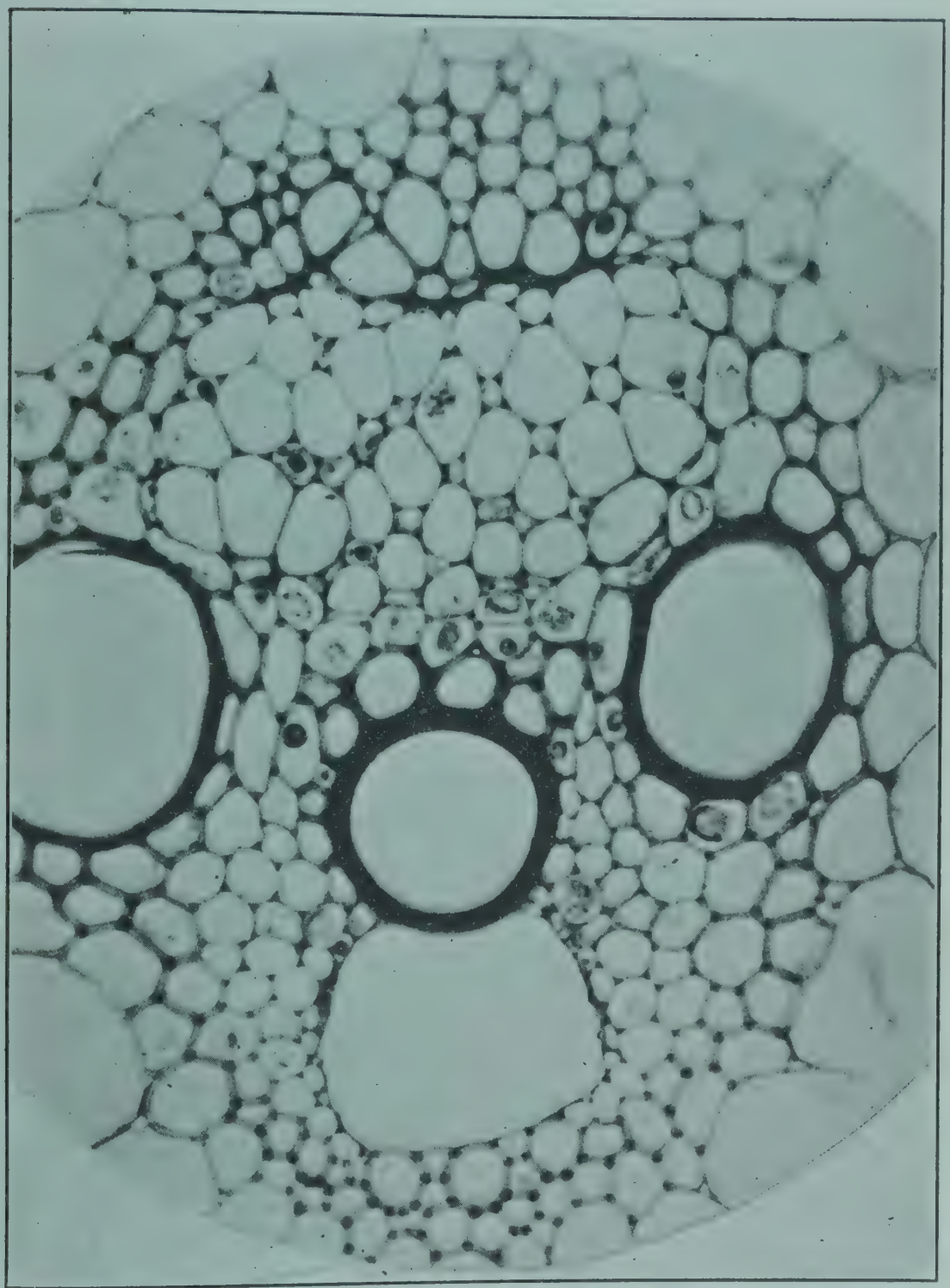


A.—Cross section through the vascular region of a root. × 210. a, Endodermis; b, protoxylem; c, large pitted vessel; d, inner sclerenchyma sheath

B.—Enlarged view of part of Figure A, to show especially the protoxylem and phloem and their relation to each other. × 510. a, Endodermis; b, pericycle; c, interstitial parenchyma; d, phloem; e, protoxylem



A.—Cross section of young root taken slightly above base of root tip. × 200
B.—Cross section of a small lateral root of a seedling. Notice that there are only three proto-xylem groups; that a pith is wanting; and that the endodermis is strongly developed. × 510



Cross section through a bundle of a small but mature corn stem. Compared with the bundle of the sugar cane, the former has better developed phloem; more pronounced obliteration of the protophloem and a larger protoxylem lacuna. The cells of the sheath abutting on the protophloem are much larger in comparison with the other sheath cells. × 480

REGARDING THE POSSIBLE ADAPTATION OF SOY BEAN RADICICOLA TO A SPECIFIC HOST VARIETY [1]

By Alfred T. Perkins,[2]

New Jersey Agricultural Experiment Station

INTRODUCTION

It has been noted by several investigators that the number of nodules formed on the roots of soy beans vary in different varieties. J. H. Vorhees [3] reported a case where Haberlandt soy beans failed to produce nodules, even though they were growing with their roots intertwined with those of other varieties which produced nodules. Later experiments have shown that Haberlandt soy beans will produce nodules, but usually to a less extent than other varieties. The question naturally arises as to what this difference in nodulation may be attributed. Theoretically, there are at least two possibilities: First, the bacteria might become adapted, in part at least, to the host variety with which they had been growing in symbiotic relationship; second, the specific host varieties of soy beans might possess different physiological characteristics which would tend to render the several varieties immune to infection in different degrees.

EXPERIMENTAL DATA

Assuming that it is possible for the bacteria to become partially adapted to a specific host variety, the author grew a culture obtained from a single bacterial cell and inoculated Virginia, Haberlandt, Mammoth Yellow, and Wilson soy beans with it. As soon as the inoculations were made, the seeds were planted in fertilized, washed sand free from nodular organisms. When nodules appeared new isolations were made, and the cultures were inoculated back upon the same host variety. Isolations were again made and the cultures which had twice passed through the symbiotic relationship with the same host variety were used in the subsequent tests on the adaptation of the organism to a specific host variety. The tests were conducted in the greenhouse under carefully controlled conditions and in the field where unknown and uncontrollable factors necessarily played a part. The results of the two series of experiments agreed very well.

The tests conducted in the greenhouse were made in flats 2 feet by 2 feet by 4 inches. The flats were filled with washed sand fertilized with magnesium sulphate, monobasic potassium phosphate, calcium carbonate, and ferrous sulphate at the respective rates of 15, 30, 100, and 10 pounds per acre. The inoculations were made by applying the nodular organism directly to the sand. The moisture content of the substratum was maintained by saturating it daily with distilled water. Four flats were used, one for each culture. Each flat was divided into nine sections by stretching strings across the top, and plantings were made with Tokyo, Hollybrook, Mammoth Yellow, Wilson, Virginia, Tar Heel, Haberlandt, Guelph, and Ito San soy beans. After four weeks' growth the crops were harvested and the data on nodulation obtained. These indicate that by twice passing through the symbiotic relationship with a specific host the organism does not become better adapted to that host than to other host varieties. In every case, regardless of the source of the organism, the Mammoth Yellow soy beans produced a greater number of nodules than the other varieties tested. Likewise, Haberlandt and Ito San always produced fewer nodules than the other varieties. These two varieties nodulated approximately to the same degree. In the four cases the relative number of nodules produced by the various hosts was about the same, and no data were obtained which indicated the least adaptation of the nodular organism to a specific host variety. The data secured from the greenhouse experiments are given in Table I.

[1] Received for publication June 11, 1924; issued April, 1925. Paper No. 174 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology. This article is part of a thesis presented to the faculty of Rutgers College in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer wishes to express his appreciation to Dr. J. G. Lipman for helpful suggestions in the planning of this work.

[3] Voorhees, J. H. Variations in soy bean inoculation Jour. Amer. Soc. Agron. 7: 139–140. 1915.

TABLE I.—*Greenhouse experiments showing relative nodulation of different varieties of soy beans when inoculated with organisms isolated from several varieties* [a]

Variety planted	Number of nodules per 10 plants of soy beans when inoculated with organism isolated from—			
	Virginia	Haberlandt	Wilson	Mammoth Yellow
Tokyo	163	182	141	173
Hollybrook	215	185	241	170
Mammoth Yellow	380	421	365	415
Wilson	112	116	170	138
Virginia	98	75	113	122
Tar Heel	150	165	132	161
Haberlandt	53	45	85	63
Guelph	138	142	107	153
Ito San	75	64	51	83

[a] Plants grown for 4 weeks in fertilized washed sand.

The field tests were conducted in cooperation with the State homes at Vineland and Skillman, N. J. Soil that had not been planted in soy beans for years was selected for the plots. Acid phosphate applied at the rate of 250 lbs. per acre was the only fertilizer employed. The inoculations were made by applying the organisms directly to the seeds. The crop was harvested at approximate maturity, the roots of a number of plants being dug in order to obtain the nodulation data. The results of the Vineland experiment are reported in Table II.

The Table II data from Skillman have not been reported, but they are practically identical with those from Vineland. The field tests were limited to 12 plots each. Organisms isolated from Wilson, Haberlandt, and Mammoth Yellow soy beans were used in inoculating these varieties; uninoculated beans were also planted. Since the uninoculated seeds gave none, or at most but few, nodules, they have not been reported on. In the field tests, without exception, Mammoth Yellow produced the greatest number of nodules, Wilson an intermediate number, and Haberlandt the fewest. As in the case of the greenhouse tests, the varieties showed always the same relative ability to nodulate, regardless of the source of the organism. No data were collected which showed a tendency on the part of the organism to become adapted to a specific variety of soy beans.

TABLE II.—*Field experiments showing relative nodulation of several varieties of soy beans when inoculated with organisms isolated from the same varieties.* [a]

Variety planted	Average number of nodules per plant of soy beans when inoculated with organisms isolated from—		
	Haberlandt	Mammoth Yellow	Wilson
Haberlandt	20	18	22
Mammoth Yellow	40	45	50
Wilson	30	26	24

[a] Plants grown to approximate maturity under field conditions.

CONCLUSIONS

The soy bean Radicicola organism does not tend to become adapted to specific host varieties of soy beans.

The differences in nodulation shown by the several varieties of soy beans may be due to some physiological difference in the varieties, possibly a difference in ability to conduct carbohydrates to the roots or proteins away from the roots.

SOWING AND PLANTING SEASON FOR WESTERN YELLOW PINE[1]

By W. G. WAHLENBERG

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INTRODUCTION

In experiments with the western yellow pine (*Pinus ponderosa*) at the Savenac Nursery at Haugan, Mont., many helpful things have been learned, although it may now be seen that the first years of experimentation were not as effective as they might have been. The problem was attacked in much the same way as that of the western white pine (*Pinus monticola*), because at that time there was no means of knowing that the western yellow pine has no such decided preference for one season of sowing over another as is the case with the western white pine. For at least two seasons sowings were made in the fall and the next spring, with the purpose of watching developments of the following season and comparing the results of the fall and spring sowings. These comparisons were carefully made and they indicated advantages for both sowing seasons. Later, when several sowing dates in the fall were compared, it became evident that the time of sowing within the fall or spring season was a larger factor than season itself. It was realized that efforts should first be directed toward the determination of the optimum time to sow within each season, and that these dates could then be used for comparisons between seasons.[2]

EARLY COMPARISONS OF SPRING AND FALL SOWINGS

Plots of western yellow pine were sown on September 15, 1913, and on May 1, 1914, and observed during 1914. The germination from fall-sown seeds started in the middle of April and was 98 per cent complete before May 18, while the spring-sown seeds had not sprouted a single individual by that date. The plants resulting from the fall sowing were also better developed in most respects than those from the spring sowing. They were slightly more top-heavy, however, the ratio of tops to roots by weight being 4 per cent larger. Weights of 100 and measurements of 200 plants were taken. From these figures it appears that promptness of germination and superior development of the plants are results of fall sowing. (See Table I.)

On October 23, 1915, two beds were sown with 8,000 seeds each, and on May 6, 1916, a similar pair of beds was sown in the same way. The spring-sown beds led in germination by nearly 50 per cent and in survival at the end of the season by 12 per cent. In top-root ratio by weight the fall-sown were more top-heavy by 8 per cent. However, the value of this comparison must be discounted because of the later finding that good results can not be expected from sowing so late in the fall.

The hold-over tendency of seed, so important with western white pine, deserves mention in connection with the western yellow pine. In most cases with fall sowing the hold-over germinations are entirely absent, although 0.2 per cent of hold over has been noted. With spring sowings the tendency is greater. Although it is usually less than 1 per cent, it may amount to more than 25 per cent under certain conditions. The 28 per cent hold over which resulted from a sowing on May 21, 1919, has been attributed to the extremely dry spring that year. (See Table III.)

[1] Received for publication May 22, 1924; Issued April, 1925.

[2] The early field work for these experiments was conducted by E. C. Rogers, assisted by P. C. Kitchin. Their manuscript progress reports cover the earlier experiments cited.

TABLE I.—*Average weights and measurements of 2-year-old seedling stock, western yellow pine*

Date sown	Length of stem	Length of main root	Length of needles	Average number of rootlets, by grades, in each range of root lengths				Weight of top	Weight of root system	Total weight of plant
				Primary		Secondary				
				0.5-2.0 inches	Over 2 inches	0.5-2.0 inches	Over 2 inches			
	Ins.	*Ins.*	*Ins.*	*No.*	*No.*	*No.*	*No.*	*Gm.*	*Gm.*	*Gm.*
Sept. 15	4.0	13.9	2.8	12.8	6.7	1.3	0.05	2.05	0.42	2.47
May 1	3.3	14.1	1.9	12.7	4.4	1.0	.06	1.09	.29	1.38

TABLE II.—*Germination and development of 1-year-old western yellow pine seedlings*

Date of sowing, 1914	Total first season germination	Total loss in seed sown from damping off and sun scorch	Development of seedlings	
			Average length of 200 stems	Average diameter of 200 stems
	Per cent	*Per cent*	*Inches*	*Mm.*
May 1	33.1	3.4	2.89	2.12
May 15	32.3	2.9	2.57	1.98
June 1	26.9	4.8	2.48	1.93

TABLE III.—*Total germination from spring sowings*

Date of sowing [a]	Percentage of seed sown germinating			
	1919	1920	1921	Total
1919				
May 5	10.0	12.7	----------	22.7
May 21	12.5	27.9	----------	40.4
1920				
Apr. 16	----------	62.2	1.9	64.3
May 5	----------	35.9	9.4	45.3

[a] 2,000 seeds sown on each date.

SPRING SOWING

In the spring of 1914 five plots of 2,000 seeds each were sown, one on May 1, two on May 15, and tw on June 1. (See Table II.) Early sowing gave a greater total germination for the first season. This appears to be due to the longer germinating period and to the favorable conditions for germination which occur during May. Early sowing appeared to favor survival slightly, although on this point the figures are not entirely in agreement. At the end of the season larger stock resulted from early sowing. In the spring of 1915 also two sowings of 6,000 seeds each were made on May 6 and May 15. Although these were but nine days apart, the total germination was again larger for the plots sown early and it was also more prompt.

Subsequent to these tests no more sowings were made and no opportunity was afforded to compare different spring sowings until 1919 and 1920, as shown in Table III. The better results in 1920 were again obtained from the earlier spring sowing, though the tendency appears to have been reversed in 1919. The unusual hold over from 1919 sowings has already been mentioned and ascribed to the dry season. The exceptional season may also be the cause of an exception to the general rule of larger totals from earlier sowings.

Both of these tests were observed in connection with a companion series sown on different dates the previous fall. In each case of spring sowing the germination was slow in starting, the bulk of it being in June and July and running through August, while the fall sowings all germinated promptly. The latter were most active in April and May and were complete by the end of June.

Seeds which do not germinate promptly in the spring, as fall and late spring sowings of western yellow pine, result in inferior plants. This has been observed both in sowing at the nursery and in direct seeding trials on planting sites. When germination takes place from the latter part of July to the end of the season, seedlings take on a characteristic abnormal appearance. At the end of the second season in the seed bed they have no fascicles of needles in twos and threes, or only one or two bunches of them. The second season's growth bears pale green straplike leaves, and the bud lacks the strong mature appearance it has in earlier-sowed plants. These inferior plants remain far behind the earlier individuals in development, and tests have shown that when field planted as 2–0 stock [3] they remain at a standstill during the first season. In all shipments of 2–0 stock it is necessary to sort out these plants. In 1915 the loss from this cause was fully 25 per cent of the stock in spring-sown beds. If sowing is done in the spring it should be as early as possible.

FALL SOWING

During 1916 to 1919 sowings were made in the fall on several dates which afford comparisons of the effect on germination of the time of fall sowing. (See Table IV and fig. 1.)

TABLE IV.—*Percentage of germination of western yellow pine seeds sown on various dates in the fall*

DATES OF SOWING [a]

1916

Germination	Aug. 21	Sept. 1	Sept. 12	Sept. 21	Oct. 4	Oct. 14	Oct. 28
Premature (fall)	3.6	4.7	0.0	0.0	0.0	0.0	0.0
Effective (next season)	32.3	30.4	39.2	43.1	50.0	48.5	41.0
Hold over (second season)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	35.9	35.1	39.2	43.1	50.0	48.5	41.0

1917

Germination	Aug. 27	Sept. 7	Sept. 17	Sept. 27	Oct. 6	Oct. 20	Nov. 3
Premature (fall)	7.0	1.0	0.1	0.0	0.0	0.0	0.0
Effective (next season)	33.3	39.4	34.2	38.9	34.6	35.9	20.1
Hold over (second season)	0.0	0.0	0.0	0.0	0.1	0.0	0.2
Total	40.3	40.4	34.3	38.9	34.7	35.9	20.3

1918

Germination	Aug. 27	Sept. 9	Sept. 21	Oct. 1	Oct. 14	Oct. 23	Nov. 4
Premature (fall)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Effective (next season)	69.7	54.5	73.1	67.7	66.8	60.7	54.8
Hold over (second season)	0.0	0.0	0.0	0.0	0.0	0.1	0.5
Total	69.7	54.5	73.1	67.7	66.8	60.8	55.3

1919

Germination	Aug. 28	Sept. 8	Sept. 16	Sept. 28	Oct. 8
Premature (fall)	0.1	0.0	0.0	0.0	0.0
Effective (next season)	54.3	46.8	40.5	46.0	37.8
Hold over (second season)	0.0	0.0	0.0	0.0	0.0
Total	54.4	46.8	40.5	46.0	37.8

[3] In nursery practice the age of plant stock is indicated by figures, the first indicating the number of growing seasons in seed beds; the second, the number in transplant beds. Occasionally a third figure is added to indicate another transplanting. Thus 2–0 indicates 2-year-old seedlings; 1–1–1 indicates 3-year-old stock, 1 year in seed beds and 2 years in transplant beds, but transplanted twice.

[a] 2,000 seeds were sown on each date.

These data for western yellow pine at Savenac Nursery indicate the following conclusions: There is practically no hold-over germination from fall sowings. When sowing is done too early in the fall, considerable "premature" germination, which is lost over winter, may result. It would be better not to sow until after the first week in September. When sowing is done too late, a marked drop in germinative capacity usually results. It would be better not to sow after October 15. Between the two extremes the results show no consistent fluctuations. The five-week period between September 7 and October 15 appears, from our present knowledge, to be a safe time to sow.

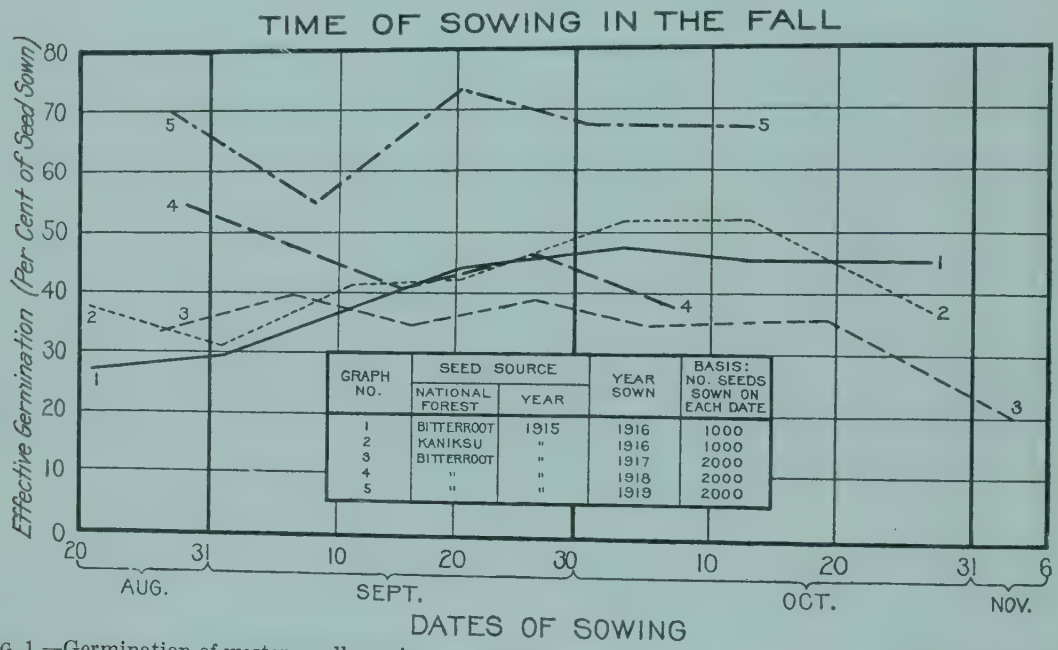

FIG. 1.—Germination of western yellow pine seed at Savenac Nursery as influenced by time of sowing in the fall

It has been observed that 1–2 yellow pine stock from fall sowings is larger, more unwieldy, and more expensive to plant than 1–2 stock from spring sowings. For the production of this class of stock, sowing should be done in the spring.

FIELD-PLANTING EXPERIMENTS WITH STOCK FROM SPRING AND FALL SOWINGS

About October 20, 1917, approximately 600 plants each of 2–0 fall-sown and 2–0 spring-sown stock were planted on a westerly aspect near Savenac Nursery. The fall sowing had been done on October 23 and the spring sowing on May 6 from the same lot of seed. At that time (1915–16) it was not realized that this date in the fall is for the average year about a week late, for best results. The effect, however, as has been shown, is to lower germinative capacity and not necessarily to affect the development of plants from the seeds which do germinate. The date of sowing in the spring seems early enough for the average season. The plants used, therefore, may be considered representative of the two classes of stock to be compared.

About April 20, 1918, this planting was duplicated. In each case the necessary precautions were taken to eliminate as far as possible all variables except the one being tested. All planting was done by the same man. The spring and fall planted rows alternated, and plants from spring and fall sown seed alternated within the rows. The plantation was examined five times in 1918 and all plants were grouped in an intensive 12-term classification. The more significant points resulting from this mass of data are reported here.

Six hundred plants from each lot would seem to be an adequate number to yield reliable averages for the test. A smaller number would often be sufficient for quite uniform stock. In order to be sure that all irregularities in the stock were actually compensating within this number, the plantation was divided into four blocks. Each block was examined and recorded separately. The comparisons within each block corresponded closely for all blocks. Therefore in order to simplify this report the tabulations are confined to data from the plantation as a whole.

TABLE V.—*Condition of 2–0 western yellow pine plantations during 1918 expressed in percentage of total number planted (600 each lot each season)*

PLANTED OCTOBER 20, 1917

Condition of the plants	Date of examination									
	June 1		June 17		July 8		July 31		Sept. 20	
	Fall sown	Spring sown	Fall sown	Spring sown	Fall sown	Spring sown	Fall sown	Spring sown	Fall sown	Spring sown
Vigorous	37.7	62.5	38.2	62.5	16.0	34.5	12.5	25.5	14.1	24.6
Failing	23.3	8.0	17.1	7.2	2.9	1.3	1.5	.5	1.2	.5
Dead	9.1	4.0	22.4	12.1	48.9	30.5	68.2	52.4	74.8	58.9
Miscellaneous—unthrifty[a]	29.9	25.5	22.3	18.2	32.2	33.7	17.8	21.6	9.9	16.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

PLANTED APRIL 20, 1917

Vigorous	91.6	90.8	89.6	90.1	50.9	51.7	37.4	37.3	36.3	32.6
Failing	2.7	1.8	1.5	2.2	.7	.5	.7	0	.3	0
Dead	.7	.7	2.5	2.3	16.0	18.9	46.4	48.2	51.6	53.0
Miscellaneous—unthrifty[a]	5.0	6.7	6.4	5.4	32.4	28.9	15.5	14.5	11.8	14.4
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] These percentages cover totals derived from nine additional plants classes which are not reported in detail here because none of them individually show any significant contrast between the behavior of spring and fall sown stock.

At the end of the first season in the field (September 20) following fall planting there were, based on fall sowing, (1) 174 per cent, or three-fourths more vigorous plants in the lot derived from spring sowing; (2) 42 per cent, or less than half as many failing plants among the spring sown; (3) 79 per cent, or four-fifths as many spring-sown plants dead as fall sown; and, on the other hand, (4) 162 per cent, or nearly two-thirds more plants unthrifty in different ways in the spring-sown lot. This last class, however, contains many plants with but slight symptoms of disorder, from which, as is indicated by the 1919 records, most of them recovered. Table V shows that these tendencies were discernible, for the most part, as early as June 1. Injury or death by accident or animal enemies was found to be small and nearly equal in the two lots, hence it does not affect the comparisons.

The second half of Table V shows the results of spring planting as they appear during the first growing season. Taken as a whole, there is very little difference between the lots from spring and fall sown seed. Both, but particularly the fall sown, have done better than when fall planted.

Figure 2 shows graphically the survival in these plantations during 1918. The steeper the curve, the more favorable the water relations, which were evidently at their worst during July. The flattening of the curves at the beginning and end of the season shows the presence of more moisture.

During 1918, also, the current height growth of the season was measured on all thrifty plants. It was as follows:

	Inch
Fall planted, fall sown, 74 plants average	0.66
Fall planted, spring sown, 125 plants average	.83
Spring planted, fall sown, 170 plants average	.76
Spring planted, spring sown, 170 plants average	.80

The spring-sown plants lead the fall sown in both cases, and this lead was greater in the case of fall planting. There was more irregularity in the spring-sown lot, due, undoubtedly, to the variation in germination dates following spring sowing.

In 1919 a simpler plant classification was used for these plantations. (See Table VI.) Again basing percentages upon the results of fall sowing, it may be seen that: (1) 143 per cent, or two-fifths more of the spring-sown than of the fall-sown plants survived when fall planted; (2) 98 per cent, or about equal survival was attained from the two lots when spring planted; (3) 134

per cent,[4] or one-third greater growth for the season was attained by the spring lot when fall planted; and (4) 82 per cent, or four-fifths as great growth was made by the spring-sown lot when spring planted.

Judging from the experiences of 1918–19, then, 2–0 western yellow pine seedlings from spring sowing survive and grow better than those from fall sowing when planted in the fall. When planted in the spring, there is practically no difference in survival as a result of season of sowing, but during the second field season the fall-sown plants lead in growth.

The question naturally arises: Why are fall-sown seedlings of this species less able to withstand the rigors of the planting site when fall planted, and yet fully the equal of spring-sown seedlings when spring planted? This leads to a consideration of the differences between plants from spring and fall sowings. Time of sowing seems to be only an indirect factor, one which is influential only in its effect on time of germination, which in turn affects plant development and hence ability to survive and grow in the field. The inferior plants resulting from late germination were described

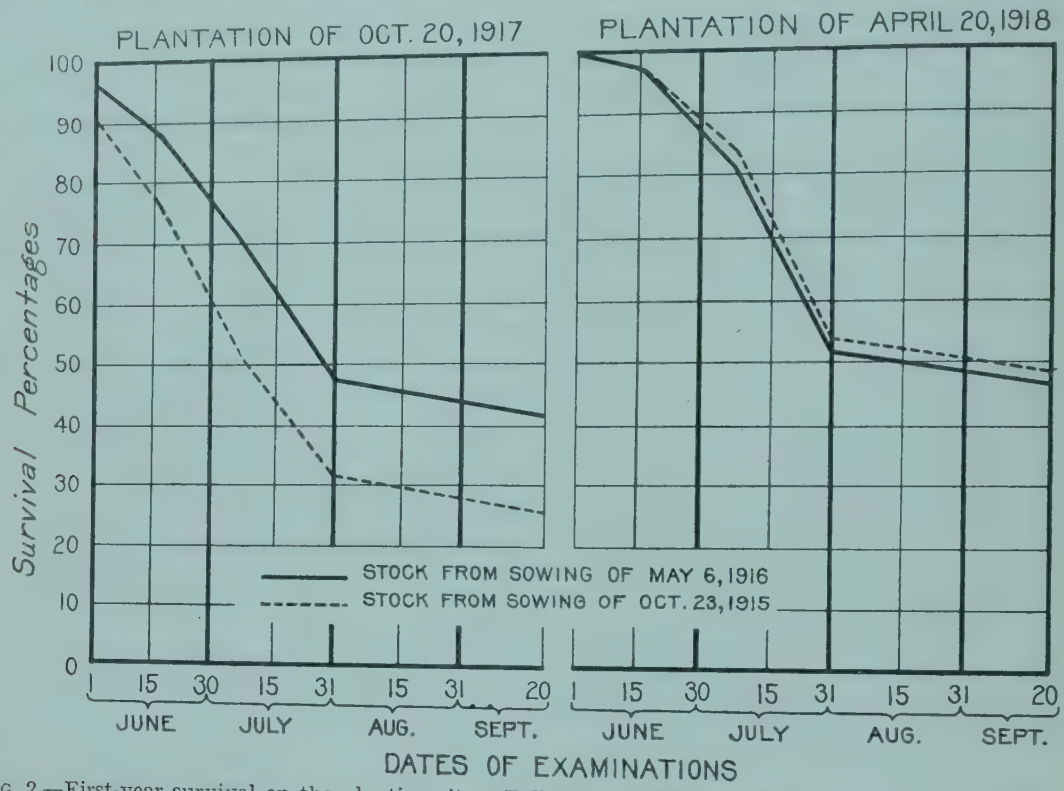

FIG. 2.—First-year survival on the planting site. Fall and spring plantings of western yellow pine 2–0 stock derived from fall and spring sowings (each curve is based on 600 plants)

TABLE VI.—*Records of the second growing season of 2–0 western yellow pine*

Season planted	Season sown	Survival percentages [a]				Growth data	
		Thrifty	Unthrifty	Total living	Total	Average stem-height growth in 1919	Number of measurements [b]
						Inches	
Fall	Spring	13.1	3.2	16.3	83.7	0.82	32
Fall	Fall	7.7	3.7	11.4	88.6	.61	32
Spring	Spring	18.1	4.2	22.3	77.7	.50	94
Spring	Fall	18.2	4.5	22.7	77.3	.61	94

[a] Figures based on examination of 600 plants in each lot.
[b] The largest common number of thrifty individuals used in determining averages.

[4] The basis for this figure is too small to insure accurate averages

under "Spring sowing." The effect of time of sowing upon time of germination has been given, and plant development, as shown in Table I, also has a bearing on the answer to this question. The ratio of tops to roots by weight was 4 per cent larger for fall-sown plants in this case; in other cases it has been found to be 8 per cent larger. The fact that fall-sown plants become more "top-heavy" is believed to be the key to the explanation sought.

When plantings are made in late October, the soil surrounding the roots remains at a temperature [5] low enough to retard water intake by any absorbing surfaces left intact by the planting act. In the meantime transpiration from the tops proceeds, the draft being made chiefly upon the moisture contained in the seedlings when planted. Gradually the saturation deficit is increased until death or a weakened condition, which handicaps the plant the following spring, results. The greater the transpiring surface in proportion to the absorbing surface present and able to function, the faster the drying process proceeds with its injurious or fatal results. Following spring planting, conditions are quite different. New absorbing surfaces are believed to develop after planting, thus preventing any long break in water absorption.

CONCLUSION

The results of these studies show that when western yellow pine is to be field planted in the northern Rocky Mountain region it is much better to use stock resulting from spring sowings for fall planting, but when spring planting is done it makes very little difference whether spring-sown or fall-sown stock be used.

When spring sowing is practiced, it should be as early in the spring as possible.

Fall sowing, if used, should be between September 7 and October 15.

Both seasons of sowing have definite advantages, but, all considered, spring seems to be the safest season as a general policy. Furthermore, spring sowing dovetails well with the general nursery work when there is a large amount of western white pine seed to be sown in the fall.

[5] From Oct. 21 to 27, 1918, at a depth of 5 inches on the site used, the daily maximum soil temperature averaged 42.0° and the daily minimum 38° F.

THE QUANTITATIVE DETERMINATION OF XANTHOPHYLL BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER [1]

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INTRODUCTION

The quantitative determination of carotin has been discussed in a previous paper.[2] Methods which have been found applicable for the determination of carotin have been found to be equally suitable for the determination of xanthophyll. The greatest difference in the absorption of the two pigments is that the bands in xanthophyll are located farther toward the violet end of the spectrum than are those for the same concentration of carotin. Accurate quantitative data on the spectral transmissive properties of xanthophyll have been obtained by the Bureau of Standards and will be published soon. This paper is concerned only with the spectrophotometric and the colorimetric methods of determining xanthophyll when in solution.

THE SPECTROPHOTOMETRIC METHOD [3]

Since carotin and xanthophyll solutions are very similar as to their light-absorbing qualities, the mercury line 435.8$m\mu$ was used as in the case of carotin. The results given in Table I and in Figure 1 were obtained with different samples of xanthophyll prepared as described below.

In general, the method of preparation of the xanthophyll used in the work described in this paper is the same as that used by Willstätter and Stoll.[4] The complete details of the preparation are to be given with data on the physical properties of the pigment in a later paper. Briefly, the method is as follows: By means of acetone the xanthophyll is extracted from dried cowpea leaves. It is separated from the accompanying carotin and chlorophyll by means of methyl alcohol extractions from a solution of the pigments in petroleum ether. The xanthophyll is extracted from the methyl alcohol by means of ether, which is then evaporated to a few cubic centimeters and methyl alcohol added. The xanthophyll is then recrystallized from methyl alcohol several times. Finally, it is dissolved in chloroform and precipitated from this by the addition of petroleum ether. It is removed from the mother liquor by filtering on a hardened filter, dried in a vacuum desiccator and quickly weighed. A solution of the pigment (0.042 gm. per liter) is made at once and readings taken the same day.

The xanthophyll used to obtain the data in experiment No. 1 and in experiment No. 2 was recrystallized from methyl alcohol and then precipitated from chloroform by the slow addition of petroleum ether. The results in experiment No. 3 were obtained by preparing a sample in the same way as in experiment No. 1 and experiment No. 2. The xanthophyll from which the sample in experiment No. 3 was taken was then dissolved in chloroform and precipitated by the addition of petroleum ether, thus giving the sample for experiment No. 4. The sample in experiment No. 5 was obtained from the xanthophyll of the sample of experiment No. 4 by dissolving it in chloroform and precipitating from petroleum ether. The xanthophyll used in the preparation of the sample for experiment No. 6 was obtained by dissolving the xanthophyll of experiment No. 5 in chloroform and precipitating it therefrom by adding petroleum ether. It was assumed that dissolving the xanthophyll in chloroform and precipitating it by the

[1] Received for publication June 7, 1924; issued April, 1925.

[2] SCHERTZ, F. M. THE QUANTITATIVE DETERMINATION OF CAROTIN BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER. Jour. Agr. Research 26: 383–400, illus. 1924.

[3] All spectrophotometric data given in this paper were obtained on the König-Martens spectrophotometer at the Bureau of Standards, U. S. Dept. of Commerce.

[4] WILLSTÄTTER, R. M., and STOLL, A. UNTERSUCHUNGEN ÜBER CHLOROPHYLL; METHODEN UND ERGEBNISSE. 424 p., illus. Berlin. 1913.

addition of petroleum ether would give a purer product each time a precipitation was undertaken, but the spectrophotometric data show that this was not the case, for evidently oxidized xanthophyll precipitated along with the pure xanthophyll.

The results for experiment No. 7 were obtained by carefully recrystallizing a sample of xanthophyll five or six times from methyl alcohol and then precipitating it from chloroform by the addition of petroleum ether. The xanthophyll used was obtained from cowpea leaves and was not allowed to stand for any length of time during the purification process. The results in experiment No. 8 were obtained in a similar manner from xanthophyll which had just been extracted from the leaves. Data obtained in experiment No. 9 were gotten by a reprecipitation from chloroform and petroleum ether of the sample used in experiment No. 8.

The xanthophyll of experiment No. 7 and of experiment No. 8 gave the best spectrophotometric results and had a melting point of 174° C. Consequently, an average of the readings for these two samples has been taken as a basis for quantitative spectrophotometric determinations.

TABLE I.—*The transmittancy of xanthophyll in ether* [a]

Experiment No.	Transmittancy [b]	Milligrams of xanthophyll per liter
1	0.0046	6.72
	.0640	3.36
	.0410	1.68
	.4740	.84
2	.0254	4.20
	.0593	3.15
	.1520	2.10
3	.0215	[c] 4.20
	.0216	4.20
	.1380	2.10
	.1410	2.10
4	.0232	4.20
5	.0195	4.20
	.0198	4.20
6	.0216	4.20
	.0219	4.20
	.1490	2.10
	.1490	2.10
7	.0180	4.20
	.0181	4.20
	.1350	2.10
8	.0167	4.20
9	.0197	4.20

[a] The xanthophyll was dissolved in ether or absolute alcohol (0.042 g. per liter) and then just before the readings were made was diluted with U. S. P. ether.

[b] For 2-centimeter cell.

[c] Duplicate readings are checks on the same solution.

In determining the purity of a sample of xanthophyll, measurements made on the spectrophotometer are apparently more valuable than melting-point determinations. Melting points were always taken but often these would be apparently correct (173°–174°) and yet the spectrophotometric results would show a difference in the absorption of the different solutions. The solution which gives the greatest light absorption is here considered as the purest. It is quite easy to remove all impurities other than oxidation products from the material. Oxidation products absorb less light than pure xanthophyll, and consequently a product which contains any oxidized xanthophyll would show less light absorption. The graphs in Figure 1 indicate the presence of oxidation products in some of the samples.

In this work the xanthophyll solution (0.42 gm. per liter) which absorbs the most light is considered as being the one which most closely approximates 100 per cent pure xanthophyll, and all of the quantitative data have been based on the graph obtained from the transmittancy of such a solution.

The composite curve of solutions (the average of experiment No. 7 and experiment No. 8) of xanthophyll which gave maximum absorption is plotted in Figure 2 in order to make the data more convenient for use and to avoid confusion with the other experimental data in Figure 1. The manner of using Figure 2 is the same as described in the paper on carotin.[5]

THE COLORIMETRIC METHOD

The colorimetric method of determining the amount of xanthophyll in solutions is similar to that described for carotin. Pure xanthophyll was prepared and 0.042 gram was dissolved in a liter of ether. Colorimetric determinations were made at once on various dilutions of this solution. Three different weighings and determinations (Table II) were made on as many different days, using Lovibond slides and a Duboscq colorimeter.

The graphs in Figure 3, which are the average results of three determinations, show clearly how the colorimeter readings obtained from Lovibond slides 5, 10, and 20 yellow, vary with the concentration of the ether solution of xanthophyll. The curves shown here are used in the quantitative work upon which the interpretation of all

[5] SCHERTZ, F. M. THE QUANTITATIVE DETERMINATION OF CAROTIN BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER. Jour. Agr. Research 26: 383–400, illus. 1924.

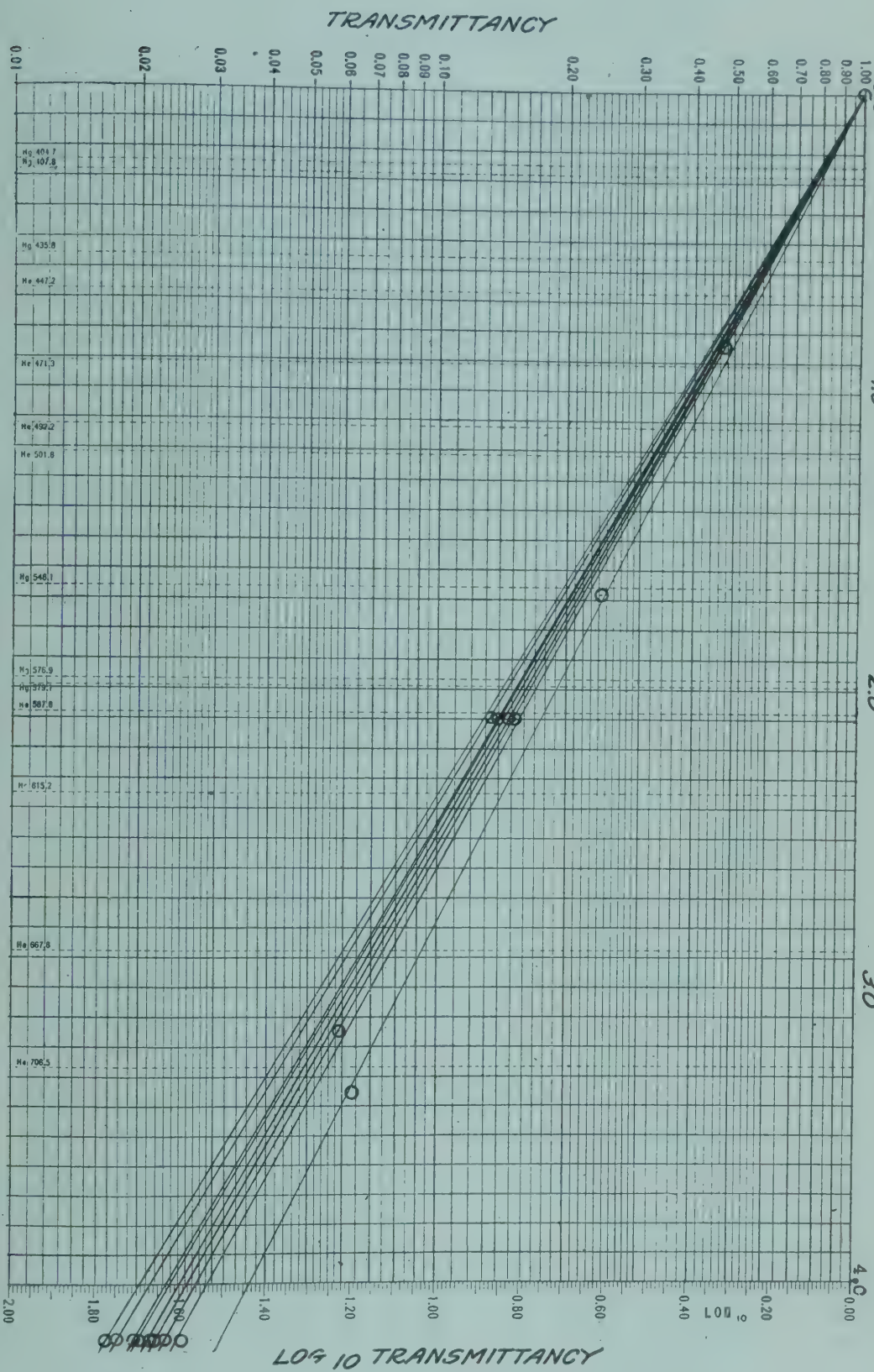

FIG. 1.—Spectrophotometric graphs obtained from ethereal solutions of different preparations of xanthophyll. Prepared from data in Table I

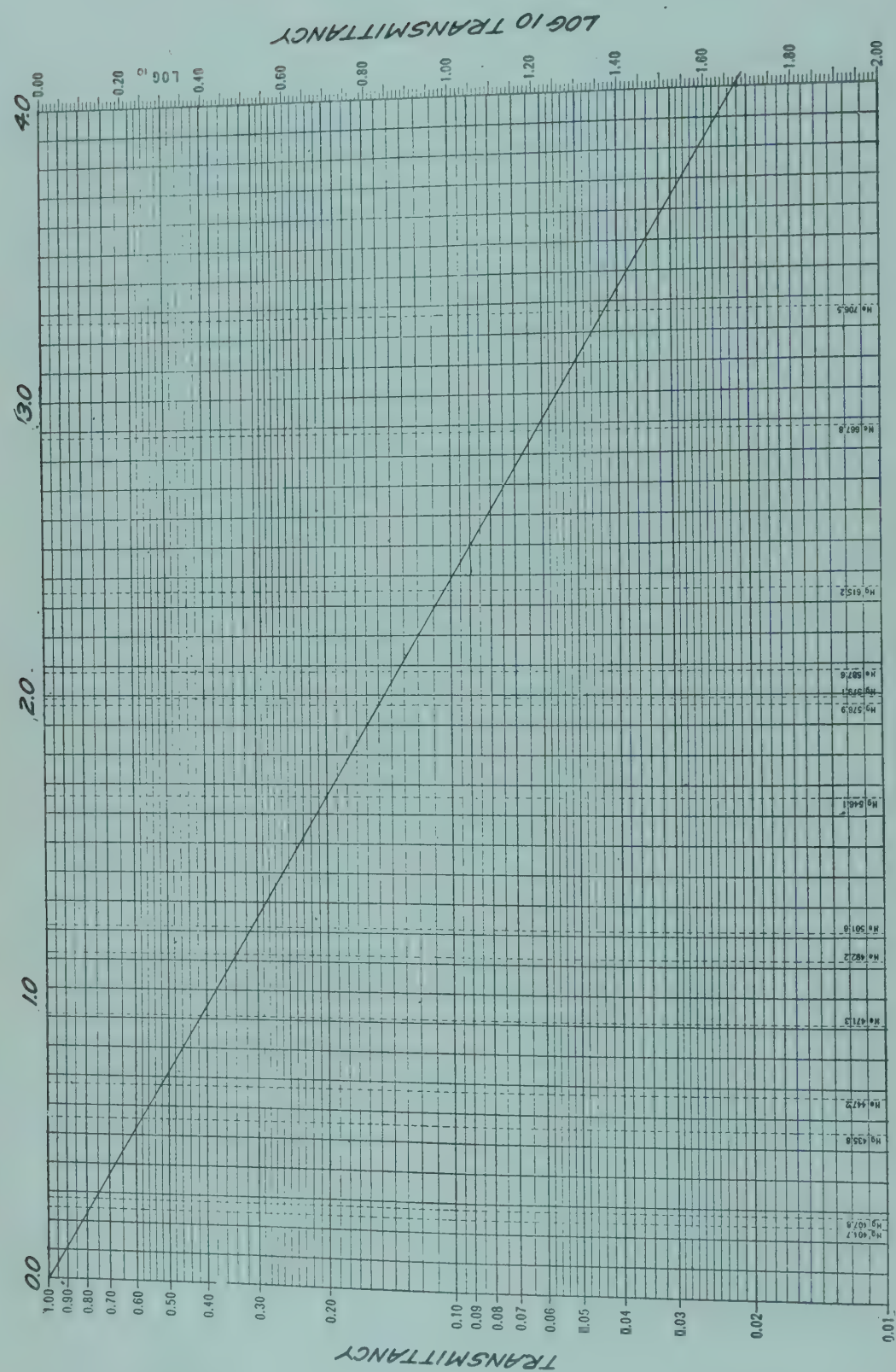

FIG. 2.—Graph of the transmittancy of an ether solution of xanthophyll, using the mercury line 435.8mμ For use in quantitative determinations

of the colorimetric readings for xanthophyll are based. The chart is used in a manner similar to the chart for carotin in the paper[6] on carotin and consequently needs no explanation.

TABLE II.—*Lovibond slide readings of an ether solution of xanthophyll*

Lovibond slide yellow	Experiment No.	Concentration of xanthophyll in milligrams per liter						
		42.0 mm.	33.6 mm.	25.2 mm.	21.0 mm.	16.8 mm.	8.4 mm.	4.2 mm.
5	1	0.60	0.80	1.1	----	1.5	2.9	6.1
	2	.50	.80	----	1.3	1.6	2.9	5.6
	3	.60	.75	.9	----	1.5	2.9	5.8
10	1	1.00	1.30	1.7	----	2.7	5.2	10.2
	2	1.10	1.30	----	2.00	2.4	5.2	9.7
	3	.90	1.20	1.6	----	2.3	4.8	9.5
20	1	1.60	1.90	2.8	----	4.1	8.0	17.4
	2	1.70	2.10	----	3.3	4.3	8.5	15.9
	3	1.70	2.10	2.9	----	4.1	7.7	15.6
Average	5	.56	.78	1.0	1.30	1.53	2.90	5.83
	10	1.00	1.26	1.65	2.00	2.46	5.06	9.80
	20	1.66	2.03	2.85	3.30	4.16	8.06	16.30

COMPARISON OF SPECTROPHOTOMETRIC AND COLORIMETRIC METHODS FOR THE QUANTITATIVE DETERMINATION OF XANTHOPHYLL

In the former paper on carotin determination it was shown that the spectrophotometer is superior to the colorimeter.

The two methods were compared also in the case of xanthophyll. A solution of xanthophyll in alcohol was used for the test. On September 6 and 7, both clear days, readings (Table III) were taken on the colorimeter each hour of the working day (fig. 4). Similar readings (Table IV) were taken on September 11, using the spectrophotometer. The 2-centimeter cell of the spectrophotometer was refilled for each reading and the solution was diluted 20 times, using ether. The results on the colorimeter, using the solution undiluted, showed a variation of 61 parts in 358, or 17 per cent while the spectrophotometric results, even after the solution used was diluted 20 times, showed a variation of only 10 parts in 354 or 2.8 per cent.

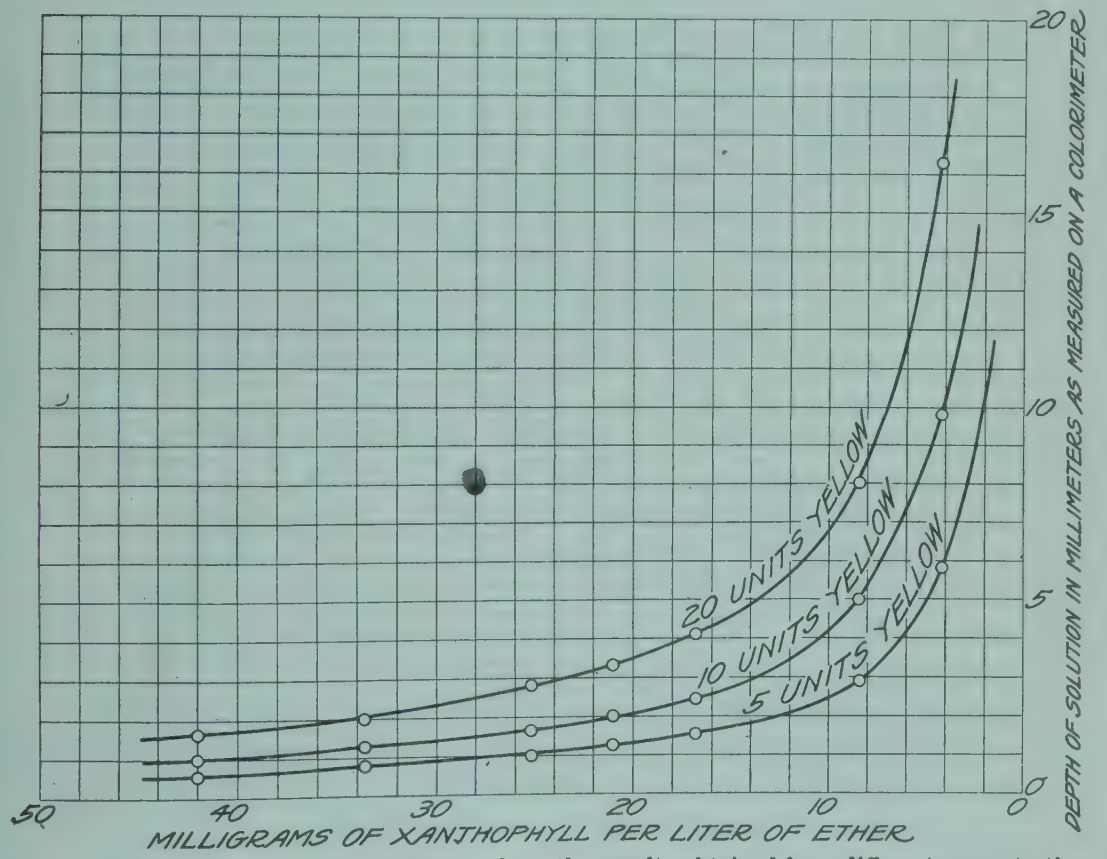

FIG. 3.—Lovibond slide readings plotted to show the results obtained from different concentrations of xanthophyll in ether

The reading in millimeters on the Duboscq colorimeter are plotted on the Y-axis while milligrams of xanthophyll per liter of ether are shown on the X-axis.

[6] SCHERTZ, F. M.—THE QUANTITATIVE DETERMINATION OF CAROTIN BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER. Jour. Agr. Research 26: 383–400, illus. 1924.

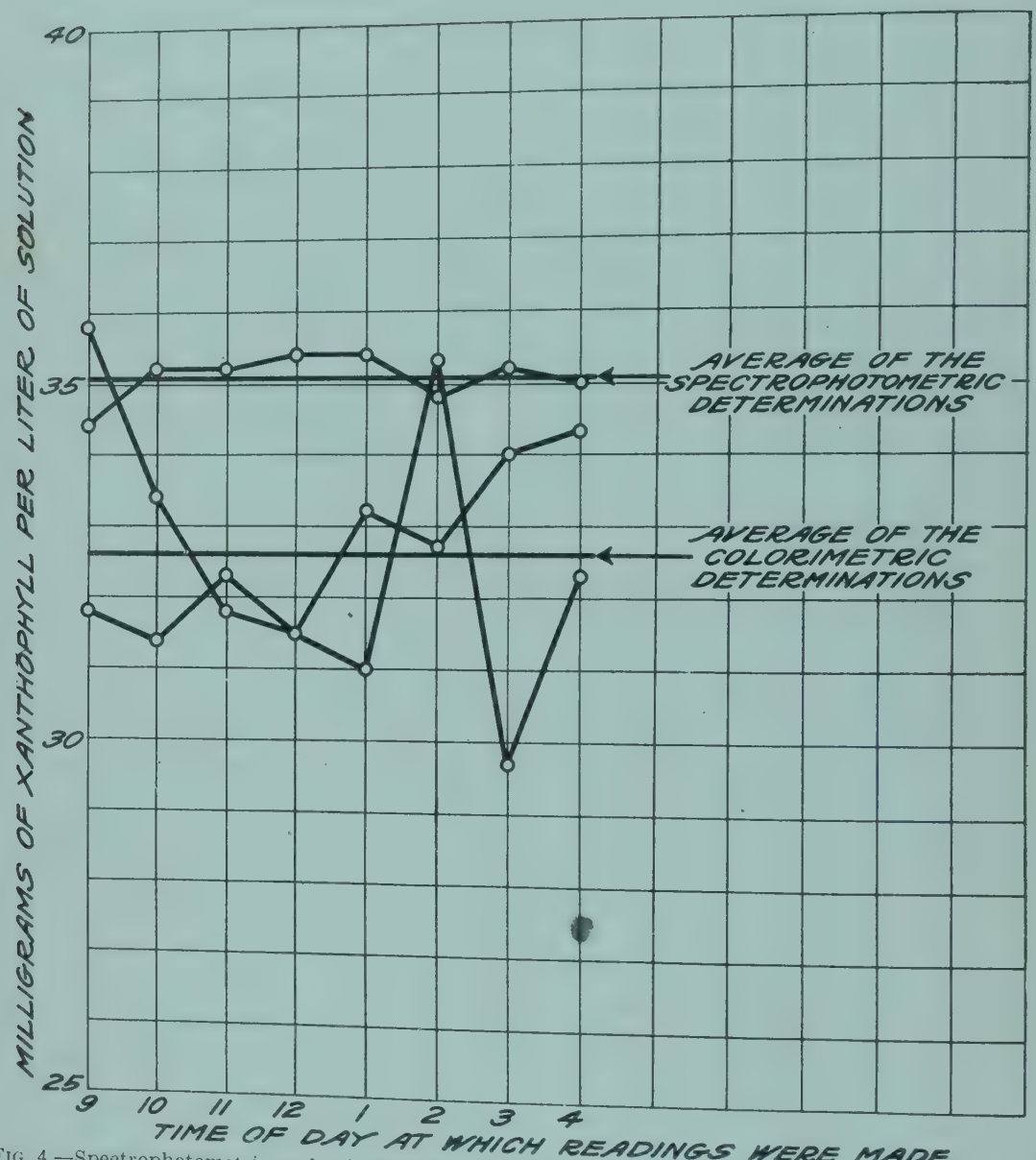

FIG. 4.—Spectrophotometric and colorimetric determinations, made at different times during the day, plotted to show the variations in the results by the two methods. The upper graph shows the results on the spectrophotometer, while the two lower graphs show the results for the colorimeter. The average for each method is also plotted

TABLE III.—*Colorimetric readings showing determinations made on the same solution at different times of the day*

Date and time of day	Depth in mm. when compared with Lovibond slides			Lovibond slide readings in terms of milligrams of xanthophyll per liter of solution			Milligrams of xanthophyll per liter of solution
	5	10	20	5	10	20	Average
September							
6, 9 a. m.	0.8	1.3	1.7	33.2	33.0	41.2	35.8
7, 9 a. m.	.9	1.3	2.2	30.4	33.0	32.0	31.8
6, 10 a. m.	.9	1.3	1.9	30.4	33.0	36.8	33.4
7, 10 a. m.	.9	1.3	2.3	30.4	33.0	30.8	31.4
6, 11 a. m.	.9	1.3	2.2	30.4	33.0	32.0	31.8
7, 11 a. m.	.8	1.3	2.3	33.2	33.0	30.8	32.3
6, 12 m.	.9	1.4	2.1	30.4	30.6	33.4	31.5
7, 12 m.	.8	1.4	2.3	33.2	30.6	30.8	31.5
6, 1 p. m.	.8	1.3	2.1	33.2	33.0	33.4	33.2
7, 1 p. m.	.9	1.4	2.2	30.4	30.6	32.0	31.0
6, 2 p. m.	.8	1.3	2.2	33.2	33.0	32.0	32.7
7, 2 p. m.	.7	1.2	2.1	37.2	35.4	33.4	35.3
6, 3 p. m.	.8	1.2	2.1	33.2	35.4	33.4	34.0
7, 3 p. m.	1.0	1.3	2.4	26.6	33.0	29.6	29.7
6, 4 p. m.	.8	1.3	1.9	33.2	33.0	36.8	34.3
7, 4 p. m.	.9	1.3	2.1	30.4	33.0	33.4	32.3

TABLE IV.—*Spectrophotometric readings showing the determinations on the same solution as that used in Table III, at different times of the day. (2-centimeter cell and mercury line 435.8 mμ used)*

Time of day	Transmittancy	Dilution	Milligrams of xanthophyll per liter of solution
9 a. m.	0.191	20 x	34.4
10 a. m.	.184	20 x	35.2
11 a. m.	.184	20 x	35.2
12 m.	.182	20 x	35.4
1 p. m.	.182	20 x	35.4
2 p. m.	.186	20 x	34.8
3 p. m.	.184	20 x	35.2
4 p. m.	.185	20 x	35.0

Such results as these obtained for xanthophyll as well as those obtained for carotin reported in a previous paper certainly militate against the use of the colorimeter for accurate work on the quantitative measurement of the plant pigments xanthophyll and carotin.

THE SPECIFIC TRANSMISSIVE INDEX OF XANTHOPHYLL

The specific transmissive index (k) for xanthophyll in ether can be calculated from the graph in Figure 2 by using the equation

$$k = \frac{-\log_{10} t}{bc}$$

TABLE V.—*The specific transmissive index of xanthophyll*

(*c*) Concentration in centigrams per liter	$-\log_{10}T$ Figure obtained from right-hand side of graph	(*b*) Thickness of cell	k
		Cm.	
0.400	1.671	2	2.089
.320	1.337	2	2.089
.220	.919	2	2.088
.140	.585	2	2.089
.100	.418	2	2.090
Average			2.089

The average value for K, the specific transmissive index, for xanthophyll in ether is 2.089 for the mercury line 435.8 *mμ* The specific transmissive index for carotin was found [7] to be 1.986 in ether, using the mercury line 435.8 *mμ* as the light source.

RELATIVE POSITION OF THE EDGES OF THE ABSORPTION BANDS (I) OF CAROTIN AND XANTHOPHYLL

Since pure pigments were available it was believed that some value might be derived from curves drawn showing the position of the edge, nearer the green, of the absorption band in a Hilger wave-length spectrometer (constant deviation type). Readings (Tables VI and VII), using a narrow slit and a 200-watt lamp as the source of white light, in *mμ* are plotted on the Y-axis in Figure 5, while the amount of carotin and xanthophyll present per liter is indicated on the X-axis. The quartz glass cells used had widths of 10, 20, and 60 millimeters. The chief value of these curves lies in the fact that they may be used in the identification of an unknown pigment as carotin or as xanthophyll.

[7] SCHERTZ, F. M. THE QUANTITATIVE DETERMINATION OF CAROTIN BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER. Jour. Agr. Research 26: 383–400, illus. 1924.

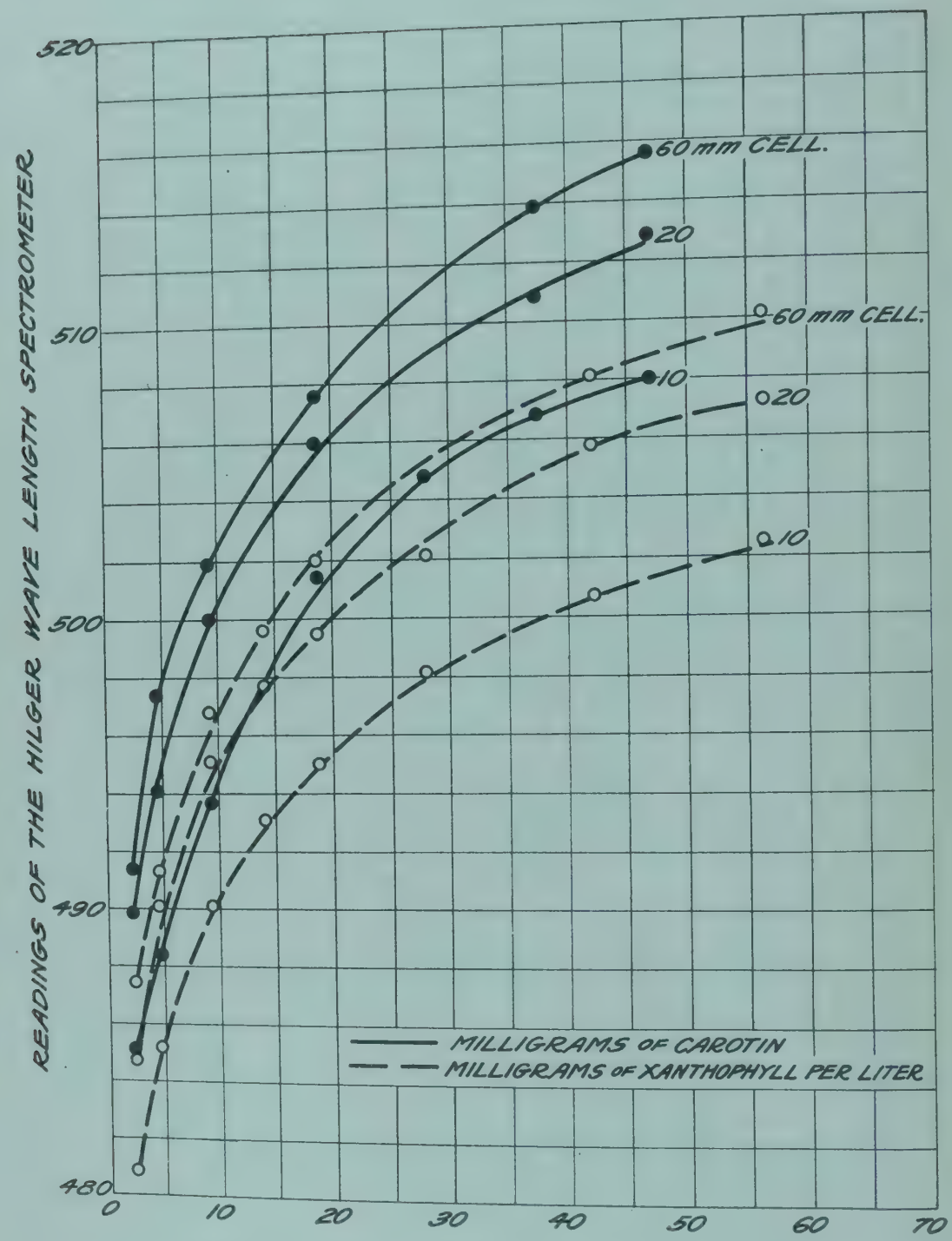

FIG. 5.—Determination made to show the relative position of the edges of the absorption bands (I) of carotin and xanthophyll

TABLE VI.—*Position of the edge of the absorption band (I) of xanthophyll in an ethereal solution*

Thickness of solution	Milligrams of xanthophyll per liter [a]							
	2.3	4.7	9.4	14.1	18.1	28.2	42.3	56.4
Mm.	mμ	mμ	mμ	mμ	mμ	mμ	mμ	mμ
10	480.9	485.2	490.1	493.1	495.0	498.1	500.7	502.5
20	484.8	490.1	495.1	497.7	499.5	502.1	505.8	507.2
60	487.5	491.3	496.8	499.6	502.0	504.8	508.1	510.1

[a] Each figure is an average of five or more settings.

TABLE VII.—*Position of the edge of the absorption band (I) of carotin in an ethereal solution*

Thickness of solution	Milligrams of carotin per liter [a]					
	2.3	4.7	9.4	18.8	37.6	47.0
Mm.	mμ	mμ	mμ	mμ	mμ	mμ
10	485.1	488.4	493.7	501.4	506.8	508.0
20	489.9	494.1	500.0	506.0	510.9	512.8
60	491.4	497.4	501.9	507.6	513.9	515.6

[a] Each figure is an average of five or more settings.

By testing the behavior of a substance suspected of being either carotin or xanthophyll, toward a mixture of petroleum ether and methyl alcohol [8] and then determining the amount of pigment present per liter by use of the spectrophotometric or colorimetric graphs (figs. 2 and 3), in connection with the graphs (fig. 5) showing where the edge of the band should come, it is easily possible to distinguish carotin from xanthophyll. Solutions of carotin and solutions of xanthophyll of known concentrations were taken and it was found that it was possible to distinguish them by means of Figure 5 alone, that is, by the position of the edge of the absorption bands. It may also be possible to tell whether a certain yellow solution contains carotin or xanthophyll, or neither.

SUMMARY

Graphs are given for various concentrations of xanthophyll in comparison with Lovibond slides 5, 10, and 20, yellow. From these graphs the amount of xanthophyll in a solution may be determined.

The transmittancy of xanthophyll in ether has been determined for the mercury line 435.8 mμ. A graph has been drawn from which it is possible to determine very accurately the amount of xanthophyll in an ether solution.

The specific transmissive index for xanthophyll in ether solution is 2.089, while that for carotin was found to be 1.986. The mercury line 435.8 mμ was used in the determinations.

Data have been submitted which show that the spectrophotometer is accurate to 10 parts in 354 or 2.8 per cent, while the colorimeter is accurate to 61 parts in 358 or 17.0 per cent.

A graph (fig. 5) has been made showing the relative position of the edge of the absorption band (I) for carotin and for xanthophyll. These may be used to ascertain whether a solution contains carotin or xanthophyll, after the concentration has been determined.

[8] Carotin will separate in the petroleum ether layer while xanthophyll will be found in the methyl alcohol layer.

CONTRIBUTION TO THE CHEMISTRY OF DECOMPOSITION OF PROTEINS AND AMINO ACIDS BY VARIOUS GROUPS OF MICROORGANISMS[1]

By SELMAN A. WAKSMAN and S. LOMANITZ

New Jersey Agricultural Experiment Station

INTRODUCTION

Extensive work has been done on the decomposition of proteins and their derivatives by microorganisms, especially by bacteria and fungi. In the great majority of cases one of the final products, ammonia, has been used as a criterion for the course and rapidity of this decomposition; witness only the large number of contributions to the so-called "ammonification" studies, both by crude and by pure cultures of microorganisms, in investigations dealing with soil microbiology. Whenever bacterial metabolism has been studied, especially nitrogenous metabolism, ammonia has usually been the product to be measured, some authorities laying more emphasis upon this as an index of bacterial decomposition of proteins (*12*)[2] and some less (*5*). Very little attention has ordinarily been paid to the transformation of the carbon part of the protein molecule or to the transformation of the available carbon present in the medium, in addition to that of the protein molecule; at least not to such an extent as to indicate the importance which this transformation plays, not only in connection with the formation or accumulation of ammonia, but with the decomposition of the protein molecule.

The purpose of this contribution is to indicate how the structure of the particular amino acids and of various proteins consisting of different amino acid groupings influences the mechanism of their decomposition by representatives of the three different groups of microorganisms, which play an important part in the decomposition of proteins in nature—the bacteria, actinomycetes, and fungi or molds.

LITERATURE

The three methods most commonly used for measuring the rapidity and amount of decomposition of proteins can be summarized as follows:

Disappearance of original protein, as in the liquefaction of gelatin, coagulation and liquefaction of milk casein, zone formation on casein agar plate, etc.—Very little attention has been paid, however, to the actual determination, in an accurate quantitative manner, of the amounts of protein decomposed. Even the numerous gelatin liquefaction studies are more qualitative than quantitative in nature. An approach has been made to quantitative methods only by the introduction of measurements of viscosity in the study of gelatin liquefaction (*30*); but even here, no differentiation has as yet been made between the mere change in the physical condition of the gelatin and its actual chemical hydrolysis, since liquefaction of gelatin may be brought about by enzymes (gelatinases), and this action may be entirely independent of proteolytic activity. Berman and Rettger (*1*) pointed out that the ability of an organism to liquefy gelatin is no sure indication of its proteolytic properties.

Formation of intermediary products in the decomposition of proteins, chiefly amino compounds.—Berman and Rettger (*1*) used the biuret test for following the course of protein hydrolysis. Sears (*25*), Waksman (*33*), and others used the Van Slyke method for determining the amino acid nitrogen as a method for following the course of decomposition of proteins. Itano (*9*), Kendall (*13*), Berman and Rettger (*1*) and others used the Sörensen (*26*) formol titration method; while DeBord (*5*) used the Folin (*8*) method for determining amino nitrogen. The determination of amino nitrogen as an index of decomposition of proteins should be used, however, only when the particular processes carried on by the different organisms are properly understood. When the protein is present as the only source of carbon and nitrogen in the medium and has to be used by the pure or mixed culture, as a source of both nitrogen and energy, the fact that the mere hydrolysis of

[1] Received for publication May 1, 1924; issued April 1925. Paper No. 167 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

[2] Reference is made by number (italic) to "Literature cited," p. 280.

the protein will liberate only a small amount of energy must be considered; the organism has to decompose the amino acids and other nitrogen derivatives of the protein molecule, and different organisms may attack different amino acids with a different degree of rapidity.

The amino compounds are therefore formed and decomposed; they will accumulate in the medium only if the particular organism is unable to use these compounds or the larger part of them, formed from the protein; they will disappear only when the organism attacks them as readily as it does the protein or when another organism is present that attacks these compounds as soon as they are formed by the other organism from the protein molecule. It would be expected that different organisms will not only differ in the rapidity and completeness with which they are able to hydrolyze one protein, and especially different proteins, but also in the kind of amino acids which they are able to utilize as sources of carbon and nitrogen. Taylor (*27*), for example, found that *Bacterium coli* did not produce amino acids from casein, but *Proteus vulgaris* produced lysin and histidin. When an available carbohydrate is present in the medium, the organisms may prefer this as a source of energy and attack the protein only as a source of nitrogen; they may, however, prefer the protein to the carbohydrate also as a source of energy. This is the reason for the various discrepancies reported in the literature on the sparing action of carbohydrates. When the carbohydrate is utilized in preference to the protein as a source of energy the latter is decomposed only to a limited extent, according to the nitrogen requirements of the organism; therefore the ammonia and perhaps even the amino nitrogen may not accumulate and may even diminish. When the protein is preferred as a source of carbon to the available carbohydrate, the amino nitrogen and ammonia may accumulate.

There is another possibility when an organism prefers only certain amino acids as sources of nitrogen, that it will decompose large quantities of protein to enable it to obtain the particular amino acids, leaving the others to accumulate in the medium. Thus the amount of amino nitrogen formed from the decomposition of proteins may fluctuate, indicating that it is continuously formed and decomposed by the organisms, as shown by Sears (*25*); or steadily increasing concentrations may be obtained for strong proteolytic organisms, as in the case of *Bacillus subtilis* and *B. pyocyaneus*. Waksman (*33*) found that the *bacterium B. coli* gave an increase of amino nitrogen in plain broth while the fungus *Aspergillus niger* showed a steady decrease. DeBord (*5*) found that the presence of glucose in peptone media increases the rate of production of amino nitrogen in cultures of various bacteria.

Use of ammonia as an index of decomposition of proteins and their derivatives by microorganisms.—One advantage of this method consists in the fact that ammonia is the end product of protein transformation and as such it is not transformed further by heterotrophic microorganisms unless there is an available nonnitrogenous substance present as a source of energy. The great advantage of this method is that the largest part of the ammonia accumulates in the medium not as a result of the nitrogen metabolism of the organism, but as a result of its carbon metabolism, when proteins and amino acids are used by microorganisms as sources of carbon, both as a source of energy and for structural purposes; ammonia is then left as a waste product of respiration of the microorganisms. This is true for both bacteria and fungi and has been well recognized by the early bacteriologists, such as Marchal (*17*). The process of ammonia accumulation has been used most extensively in the literature of microbiology as an index of protein decomposition, especially so in the branch of the subject applied to soils, because of the difficulty of extracting and determining residual proteins or intermediary products in the soil as a medium, and the ease of ammonia determination. The term "ammonification" has come into general use, although the term "ammonization" would be the more proper one, since it came first into use in the nineties of last century, when suggested by Marchal.

According to Butkevitch (*3*), ammonia formation is not the final stage in respiration, but the first stage, since ammonia is first split off (deaminized) from the amino acid, leaving the remaining group available as a source of energy for the organism.

Ammonia accumulation can serve as a good index of proteolysis only when no available carbohydrates are present; in the presence of the latter this method should be supplemented by the study of another process, either the disappearance of original protein, or the formation of amino nitrogen. This accounts for the fact that Kendall and Walker (*12*), Butkevitsch (*3*), Marchal

(*17*) and numerous other investigators found ammonia formation a good index of proteolysis, while DeBord (*5*) and others found that it is not a reliable index. The term is merely relative and should not be made to mean any more than it does.

When a protein is acted upon by microorganisms it is first hydrolyzed into various protein derivatives, including the amino acids; the latter are then deaminized. It is important to know whether all constituents of the protein molecule are acted upon alike by microorganisms, or whether some are acted upon first and others after. Robinson and Tartar (*22*) found that all the nitrogenous compounds are changed more or less by the bacteria with the formation of ammonia; in no case was one of the derivatives completely destroyed, but the rapidity of action varies with the different proteins. Lathrop (*15*) pointed out that the monoamino and diamino acid nitrogen (especially the latter, according to Kelley (*11*)) are the chief sources, in the protein molecule, from which ammonia is formed.

There are available, however, undisputed facts in the literature that certain organisms prefer one chemical group to another and that different organisms may vary in their preferences in this respect. Rubner (*23, 24*) demonstrated calorimetrically that bacteria consume a great deal more material for energy than for growth (increase in protoplasm). *B. proteus*, for example, utilized, in a period of 10 days, 3.01 calories for growth, out of 12.32 calories transformed; longer periods of growth even increased the ratio of the number of calories used for energy to that used for growth. Growth and energy consumption are so related that they are affected alike by environmental conditions, as temperature. Different species, however, were found by Rubner to behave differently in this respect.

The fungi producing an abundant growth and assimilating a large amount of the available carbon behave differently in this respect; about half of the nutrient consumed is used for resynthesizing the cells (*14*); Terroine and Wurmser (*28, 29*) found that *Aspergillus niger* stores in the mycelium as much as 59.6 per cent of the metabolizable energy. It may thus be concluded that, as a general rule, bacteria utilize a much smaller amount of energy than fungi for growth and the synthesis of protoplasm.

As to the decomposition of different amino acids and acid amides in the soil, which contains a mixture of a large number of organisms, Jodidi (*10*) demonstrated that the rate of their transformation into ammonia is greatly influenced by their chemical structure; amino acids and acid amides of equal structure were found to yield about the same proportion of ammonia.

According to Blanchetière (*2*), alanine, leucine, aspargine, phenylalanine, tyrosine, and histidine are readily utilized by *Bact. fluorescens liquefaciens*, as sources of both carbon and nitrogen; glutamic acid and tryptophane are utilized more slowly, and glycocoll only after a period of incubation, longer than one month.

EXPERIMENTAL DATA

A standard medium, synthetic in composition, has been used in the following investigations; it contains the following mineral constituents:

K_2HPO_4	1.0 gram
$MgSO_4 .7H_2O$	0.5 gram
NaCl	0.1 gram
$FeSO_4$	0.02 gram
Distilled water	1,000 c. c.

One per cent of the various proteins and amino acids was added to the above medium. Pure or anhydrous dextrose was used in 2 or 1 per cent concentrations. The amino acids were purified commercial products and consisted, therefore, of the racemized forms. The casein was purified according to Hammarsten and brought into solution, by dissolving 1 gm. of casein in 8 c. c. of 0.1N NaOH then adjusting the reaction with HCl. The media were placed, in 100 c. c. portions, in 250 c. c. Erlenmeyer flasks, plugged with cotton and sterilized at 10 pounds for 30 minutes. The media were then inoculated with the various organisms grown on agar slants.

The following organisms have been used in this study:

Trichoderma koningi Oud. and *Zygorhynchus mölleri* Vuill. isolated from the soil and kept in culture for some time.

Actinomyces viridochromogenus (Krainsky) Waksman isolated from the soil in 1916 and described in detail elsewhere.

Bacillus cereus Frankland isolated from the soil.

Bacterium fluorescens (Flügge) Lehm. et Neum. isolated from the soil.

The following procedure of analysis was used. At the end of the period of incubation the cultures were filtered through weighed pieces of ashless filter paper and the mycelium washed with a little distilled water so as to bring the filtrate to original volume. The mycelium was then dried to constant weight at 65° to 85° C., then analyzed for

total nitrogen by the Kjeldahl method. The ammonia nitrogen was determined in an aliquot portion (25 c. c.) by the Folin (*7*) aeration method, using 5 c. c. of 40 per cent Na_2CO_3 solution and some heavy oil, to prevent foaming, and aerating, for 4 hours, into $\frac{N}{10}H_2SO_4$, then titrating. The amino nitrogen of the liquid was determined by the Van Slyke micro method (*31*, *32*), after the ammonia had been aerated, or, in the original solution, allowing for the ammonia content by subtracting one-half of the ammonia nitrogen from the total amino nitrogen (shaking 5 minutes). The total nitrogen in the solution was obtained by determining it in an aliquot portion of the filtered culture. The residual dextrose was determined by the Bertrand method. The hydrogen ion concentration of the medium was determined by the colorimetric method. In view of the fact that, in the case of the bacteria, the mere filtration through paper gave the weight of the bacterial pellicle but not of the cells throughout the medium, attempts have been made to obtain the amount of the latter by carefully acidifying with acetic acid, then filtering the coagulated mass. This process has been carried out successfully only in the case of the bacteria growing on glutamic acid media. The total weight of the bacterial cells, in the other culture, is therefore only approximate and indicates merely the weight of the pellicle. In the case of the fungi and the Actinomyces, however, filtration through paper is sufficient to separate the growth from the culture fluid completely.

In all cases duplicate cultures were prepared, using an uninoculated control for every fresh lot of medium; only the averages of the two determinations are recorded; in those cases where the results in the duplicates varied markedly they were reported separately. Several of the experiments were repeated, but unless markedly different results were obtained (as in the case of the 1.4 per cent glutamic acid) they were not recorded.

Before discussing the various chemical changes produced by the different microorganisms it may not be out of place to point out several outstanding characteristics in the growth of these organisms. The *Bact. fluorescens* used the carbon and especially the nitrogen of the various amino acids in a most excellent way, producing a heavy pellicle on the surface, especially so in the presence of dextrose; the characteristic fluorescent pigment was produced in most cases on the second or third day of growth only in the amino-acid media not containing any dextrose. The *B. cereus*, however, made no growth at all with glycocoll, alanine, and phenylalanine and only very little growth with glutamic acid and asparagine, which indicates that this organism is not only incapable of utilizing the carbon in at least the amino acids tested, but can not even make use of their nitrogen. Such a distinctive difference between these two very common groups of soil bacteria, one (the *Bact. fluorescens*) a nonspore-forming organism and the other (*B. cereus*) a spore former, was very unexpected. When these two organisms were inoculated into the synthetic medium containing purified casein, the exactly reverse phenomenon took place —the *B. cereus* developed very rapidly, accompanied by a vigorous decomposition of the casein, while the *Bact. fluorescens* made only a mere trace of growth without bringing about any noticeable hydrolysis of the casein. Thus one organism proves to be strongly proteolytic, capable of rapidly hydrolyzing proteins (this was found to hold true also for purified vegetable proteins, as will be shown later), but unable to attack some simple amino acids; the other is unable to attack native proteins, but is capable of making a very abundant growth on the protein cleavage products—the amino acids.

In view of the fact that these two organisms do not occur in nature in pure culture but in constant association, and the further fact that one can readily utilize the products of the other, the idea suggested itself that proteins could more rapidly be reduced to the ammonia stage by the combined action of these two organisms, one utilizing the products of the other. This has actually been found to be the case, as will be pointed out later.

Another interesting phenomenon was observed in the case of the fungi. Whenever pure amino acids have been used as the only source of carbon and nitrogen, the fungi made a rather limited growth, as shown in Tables I to IV; in most cases not over 50 mg. per 100 c. c. of medium. One could, therefore, be led to a hasty conclusion that fungi can not readily utilize amino acids as a source of carbon. On close examination of the data, however, a more appropriate explanation of this phenomenon is found. In all cases whenever amino acids have been used as sources of carbon and nitrogen, the reaction is changed to alkaline, owing to the rapid accumulation of ammonia. In view of

the fact that the initial reaction of the medium was about pH 7.0 to 7.3 and the medium is not very highly buffered, the reaction changed rapidly to pH 8.0 and even 8.6, which seems to be about the limiting reaction for the growth of at least the two fungi studied. In one case (with 1 per cent glutamic acid) the reaction was left by mistake unadjusted at a pH of 3.0. The bacteria and Actinomyces made no growth, since the reaction was too acid for their development, but the two fungi made a very excellent growth. This shows that there are various factors to be considered in the study of utilization of organic substances under artificial conditions, especially by pure cultures of microorganisms. Notwithstanding the statements made that fungi are not affected by wide ranges in reaction, there is no doubt that, starting with a reaction of about pH 6.0 to 7.0, they will be more favored by the presence of substances which result in products tending to make the reaction acid rather than alkaline; and in the presence of alkaline-forming substances they will be more favored by an acid than by an alkaline or even a neutral reaction.

In connection with the utilization of racemic compounds by microorganisms, attention should be called to the extensive literature on this subject since the work of Pasteur. According to Ehrlich (*6*), *dl*-proline, *dl*-aspartic acid and *dl*-tyrosine are acted upon by yeasts symmetrically, whereas *dl*-alanine, *dl*-glutamic acid and others are acted upon asymmetrically. Pringsheim (*20*) as well as Neuberg (*19*) found that *dl*-glutamic acid and *dl*-aspartic acid are acted upon by fungi and bacteria symmetrically. Various other investigators have shown that, while some organisms attack some amino acids symmetrically, other organisms attack the same acids asymmetrically, depending on the nature of the compound and the organism.

DECOMPOSITION OF GLYCOCOLL (GLYCINE)

The chemical formula for glycocoll is $CH_2.(NH_2).COOH$; in other words, it contains theoretically 18.67 per cent nitrogen and 32.0 per cent of carbon, the ratio between the carbon and nitrogen being 1.7. According to Levene and Van Slyke (*16*), the amount of amino acid obtained for glycocoll, by the Van Slyke method, is somewhat higher than its actual nitrogen content; the figures obtained for amino nitrogen in the case of glycocoll should, therefore, be multiplied by 0.93 to obtain correct values. Somewhat higher results for the NH_2-N in the case of this amino acid were actually obtained in our studies and the results were adjusted by multiplying the figures by 0.93. The growth of only two organisms on glycocoll, in the absence of dextrose, is reported: Trichoderma and Actinomyces (Table I). These two organisms used up, in 12 and 15 days respectively, 29.8 and 41.1 mg. of the amino acid nitrogen and synthesized 50 and 59 mg. of dry mycelium. The Trichoderma produced ammonia equivalent to 24.28 mg. of nitrogen, or nearly 82 per cent of the amino acid decomposed; the Actinomyces produced 30.46 mg. ammonia nitrogen, or 74 per cent of the amino acid decomposed. (The figures for NH_3-N include also the NH_3-N found in the control.) The Trichoderma assimilated over 44 per cent of the carbon decomposed (assuming that the mycelium contains 45 per cent carbon), and the Actinomyces about 38 per cent.

Where dextrose is present the question of carbon and nitrogen utilization by the organisms is somewhat more complicated. The Zygorhynchus decomposed, in 6 days, 22.7 mg. of the glycocoll nitrogen; and, in 10 days, 42.1 mg. In view of the excess of dextrose, most of the nitrogen has been reassimilated by the organism (17.63 mg. out of the 22.7 mg. decomposed) and a small amount is left as ammonia; in 10 days all the dextrose has been used up, and the organism is forced to attack the amino acid molecule for its carbon, which is the reason for the more rapid decomposition and greater accumulation of ammonia. The same is true even to a more striking extent in the case of the Trichoderma. This organism has decomposed, in 5 days, glycocoll equivalent to only 48.6 mg. of nitrogen, 45.66 mg. of which has been reassimilated and resynthesized into fungous mycelium; only a trace of ammonia is found. However, after 10 days, glycocoll equivalent to 108.2 mg. of nitrogen is decomposed, 49.03 mg. is found in the mycelium and 42.80 mg. as ammonia. This is due to the fact that, after the organism has used up all the dextrose, it has to decompose the glycocoll as a source of carbon; so much so that the mycelium actually began to undergo autolysis, as indicated by the diminishing weight. The fact that the nitrogen content of the mycelium did not decrease, while the total weight did, indicates that, after all the dextrose in the medium has been used up the organism will utilize as a source of energy certain carbohydrates

TABLE I.—*The chemistry of decomposition of 1 per cent glycocoll (glycine) by microorganisms, in the presence and absence of dextrose*

Organism used	Dextrose	Age of culture	Total N in 100 c. c.		NH_3—N in 100 c. c.		NH_3—N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Decrease from control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None.	------	180.3	-------	178.1	-------	3.54	-------	-------	--------	-------	7.1
Do	2	------	180.3	-------	178.1	-------	3.54	1,870	-------	--------	-------	6.5
Zygorhynchus	2	6	162.8	17.5	155.4	22.7	4.40	380	1,490	251	17.63	6.4
Do	2	10	146.8	33.5	129.0	42.1	13.42	0	1,870	473	27 74	7.2
Trichoderma	None.	6	177.0	3.3	167.4	6.2	7.98	-------	-------	30	3.10	7.8
Do	None.	12	175.0	5.3	148.3	29.8	24.28	-------	-------	50	3.30	8.1
Do	2	5	129.7	50.6	125.5	48.6	1.32	380	1,490	804	45.56	6.7
Do	2	10	122.8	57.5	69.9	108.2	42.80	0	1,870	669	49.03	7.8
Actinomyces	None.	8	178.7	1.6	172.0	4.1	6.36	-------	-------	23	1.4	7.7
Do	None.	15	167.2	13.1	137.0	41.1	30.46	-------	-------	59	4.0	8.0
Do	2	8	157.7	22.6	135.1	36.0	18.84	1,300	570	213	17.5	7.0
Do	2	15	142.0	38.3	108.8	62.3	30.62	1,110	760	303	29.0	7.4
Bact. fluorescens [a]	None.	9	174.9	5.4	168.0	10.1	6.36	-------	-------	(?)	(?)	7.5
Do	2	5	163.1	17.2	151.8	26.3	2.56	1,050	820	99	10.86	6.6
Do	2	9	(?)	-------	137.2	40.9	7.00	340	1,530	123	18.60	6.4

[a] Pellicle only incompletely removed.

stored away in its own mycelium in preference to attacking the amino acid; possibly after half of the amino acid has been used up (the natural *d*-form) the remaining *l*-form is attacked with greater difficulty.

In the case of the two fungi, dextrose, as an available carbohydrate, had a sparing action upon the decomposition of the amino acid as well as upon the accumulation of the ammonia. The amino acid is not decomposed as a source of energy and the ammonia does not accumulate (at least not in appreciable amounts) as long as available energy, in the form of dextrose, is present. Actually a larger amount of the amino acid may be decomposed, even in the presence of dextrose; but all the available nitrogen is reassimilated by the organism, so that there is no ammonia left as a waste product.

The Actinomyces, however, decomposed the amino acid and allowed an appreciable accumulation of ammonia, even in the presence of dextrose. After 15 days' incubation, 1,110 mg. of dextrose was still left in the medium, while over 30 mg. of nitrogen as ammonia had already accumulated, owing to the fact that actinomycetes readily utilize amino acids and proteins as sources of carbon or energy; they also prefer these as sources of nitrogen to the ammonia, which accumulates in the medium, even in the presence of dextrose. In this case, then, one can not speak of any sparing action of carbohydrates over proteins, nor can one even justify any theory of "fermentation in preference to putrefaction," meaning, of course, that microorganisms utilize more readily available carbohydrates as sources of energy than they do proteins. It does apply at least to the particular Actinomyces.

The bacterium (*Bact. fluorescens*) behaved similarly to the fungi, decomposing the amino acid in the presence of dextrose only to a limited extent and allowing the accumulation of only small quantities of ammonia, as long as the dextrose lasted in the medium. Unfortunately, it was not possible to obtain the proper weight of the cells for this organism, since it made a rather scanty growth in the glycocoll medium free from dextrose. Blanchetière (*2*) found that this organism transformed 81 per cent of the amino-acid nitrogen into ammonia in 55 days, in the absence of dextrose.

The change in reaction depended entirely on the substances that were being changed. In the absence of dextrose the reaction changed, in all cases, to more alkaline, due to the formation of ammonia; in the presence of dextrose the reaction either did not change or became slightly acid; as soon as the dextrose was used up, or, in the case of the Actinomyces, with the continued decomposition of the glycocoll, even in the presence of dextrose, the reaction became alkaline.

Just how do microorganisms utilize an amino acid as a source of energy? Nawiasky (*18*) suggested the following group of reactions:

TABLE II.—*The chemistry of decomposition of 1 per cent alanine by microorganisms, in the presence and absence of dextrose*

Organism used	Dextrose	Age of culture	Total N in 100 c. c.		NH_2—N in 100 c. c.		NH_3—N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Decrease from control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None.		153.5		151.5		2.36					7.1
Do	2		153.5		151.5		2.36	1,760				6.8
Zygorhynchus	None.	12	146.0	7.5	129.4?	22.1?	6.40			48	3.86	7.8
Do	2	12	141.3	12.2	120.2?	31.3?	1.12	570	1,190	175	9.29	5.6
Actinomyces	None.	17	125.1	28.4	64.0	87.5	39.17			126	8.97	8.6
Do	2	17	144.4	9.1	105.4	46.1	6.82	1,230	530	82	7.58	4.9
Bact. fluorscens	None.	5			70.0	81.5	32.82					8.8
Do	2	5			92.8	58.7	1.72	750	1,010			4.8
Control	None.		147.6		145.3		3.40					7.3
Do	2		147.6		145.3		3.40	1,680				6.8
Trichoderma	None.	6	135.2	12.4	107.5	37.8	21.98			80	6.97	8.3
Do	2	6	124.4	23.2	107.6	37.7	7.64	400	1,280	562	24.32	5.2

(1) $R.CH(NH_2).COOH+2H=R.CH_2.COOH+NH_3$

$R.CH_2.COOH+3O=R.COOH+CO_2+H_2O$

$R.COOH=R\ H+CO_2$

The first step in the reaction is the splitting off of the ammonia (reductive deaminization), followed by the gradual decomposition of the nitrogen-free molecule, resulting in the liberation of energy and CO_2. The reaction may also take place as follows:

(2) $R.CH(NH_2).COOH=R.CH_2.NH_2+CO_2$

(3) $R.CH(NH_2).COOH+H_2O=R.CHOH.COOH+NH_3$

In reaction (2) we have the formation of an amine, as an intermediary step, and liberation of energy and CO_2. In the third reaction we again have deaminization, by means of hydrolysis, with the formation of an oxy-acid.

Nawiasky found that *B. proteus* is capable of attacking glycocoll to a limited extent, changing a part of it to acetic acid.

$CH_2(NH_2).COOH+2H=CH_3COOH+NH_3$

A part of the acetic acid is probably utilized as a source of energy, in the absence of an available carbohydrate.

DECOMPOSITION OF ALANINE

The chemical formula for alanine is $CH_3.CH.(NH_2).COOH$; it contains 15.73 per cent nitrogen and 40.4 per cent carbon, or 2.57 times as much carbon as nitrogen. The Trichoderma decomposed alanine equivalent to 37.8 mg. of nitrogen, in the absence of dextrose (Table II); out of this, 21.98 mg. has been changed to ammonia and 6.97 mg. has been reassimilated. The organism synthesized 80 mg. of mycelium, which contains about 36 mg. of carbon; the amino acid decomposed contained $37.8\times2.57=97.15$ mg. of carbon; in other words, the organism reassimilated about 37 per cent of the carbon that it has decomposed. About the same amount of alanine has been decomposed by the Trichoderma also in the presence of dextrose, but a much smaller amount of the nitrogen is found as ammonia and a much larger quantity of it was reassimilated. The process of carbon utilization in the presence of dextrose is

$$\frac{562\times45\text{ per cent}}{1{,}280\times40\text{ per cent}+37.7\times2.57}=41.5$$

per cent. The organism is somewhat more efficient in synthesizing its protoplasm, in the presence of an available carbohydrate, requires less energy, and assimilates less nitrogen per unit of protoplasm synthesized.

The Actinomyces synthesized 126 mg. mycelium from an amount of alanine decomposed which is equivalent to 87.5 mg. of nitrogen; the efficiency of this organism is $\frac{126\times45\text{ per cent}}{87.5\times2.57}=25.1$ per cent. In the presence of dextrose, the Actinomyces decomposed alanine, equivalent to 46.1 mg. of nitrogen, and 530 mg. of dextrose, but synthesized only 82 mg. of mycelium, showing a lower efficiency. This tends again to bring out that the Actinomyces may prefer

amino acids and protiens as sources of energy, even in the presence of such an available carbohydrate as dextrose.

The development of *Bact. fluorescens* in the alanine medium was very similar to its growth upon glycocoll; that is, a larger amount of amino acid has been decomposed and a much larger amount of ammonia has accumulated in the absence than in the presence of dextrose. The changes in reaction were similar to those observed with glycocoll.

In comparing the amounts of ammonia found in the medium with the amount of growth produced by the organisms upon glycocoll and alanine, as substrates, attention should be called to the following fact: The Trichoderma liberated, in the case of glycocoll, 24.28 mg. nitrogen as ammonia for 50 mg. of mycelium synthesized and, in the case of alanine, 21.98 mg. NH_3—N for 80 mg. of mycelium; the Actinomyces liberated, in the case of glycocoll, 30.46 mg. of NH_3—N for 59 mg. of mycelium and, in the case of alanine, 39.17 mg. NH_3—N for 126 mg. of mycelium. This difference is due not to the more rapid decomposition of the glycocoll (just the reverse is true), but to the fact that the C:N ratio is smaller, in the case of glycocoll than in the case of alanine; the organisms will find more available energy in the latter and will, therefore, assimilate more of the nitrogen and leave a smaller amount of waste as ammonia.

DECOMPOSITION OF PHENYLALANINE

The chemical formula for phenylalanine is $C_6H_5.CH_2.CH(NH_2).COOH$; this amino acid contains 8.49 per cent nitrogen, 65.5 per cent total carbon and about 22 per cent carbon, outside of the benzol ring. The Zygorhynchus and Actinomyces decomposed only a small amount of the phenylalanine (Table III); the color of the medium soon became reddish and the odor distinctly aromatic. It is therefore possible that the organism is unable to utilize the various products formed from the decomposition of the phenylalanine, containing the benzol group; their rapid accumulation, especially in the absence of dextrose, may soon stop the further development of the organism. The Trichoderma, however, used up, in 6 days, 37.6 per cent of the phenylalanine in the absence of dextrose and 56.4 per cent in the presence of dextrose. If the efficiency of the organism to assimilate carbon is as great with phenylalanine as with the other amino acids—40 per cent—we could conclude that the Trichoderma attacked also the benzol group; 131 mg. of dry mycelium contains about 60 mg. C, which would indicate about 150 mg. of original carbon decomposed; 37.6 per cent of the phenylalanine which was decomposed in the medium (or 338 mg. of phenylalanine) contained about 220 mg. of total carbon and only about 75 mg. of the carbon outside of the ring.

The same is true of the cultures containing the dextrose. All the nitrogen of the amino acid decomposed was recovered as ammonia and in the mycelium.

Of the two bacteria, *B. cereus* made no growth at all, while *Bact. fluorescens* decomposed the phenylalanine very rapidly, over 55 per cent in 7 days, in the absence of dextrose, thus showing

TABLE III.—*The chemistry of decomposition of 0.9 per cent phenylalanine by microorganisms, in the presence and absence of dextrose*

Organisms used	Dextrose	Age of culture	Total N in 100 c. c.		NH_3-N in 100 c. c.		NH_3-N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Decrease from control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None		79.80		73.96		Tr.					7.1
Do	2		79.80		73.96		Tr.	1,700				6.5
Zygorhynchus	None	7	77.90	1.90	66.36	7.60	8.56			35	2.4	7.7
Do	2	7	66.10	13.70	61.08	12.88	2.98	370	1,330	272	14.00	5.2
Trichoderma	None	6	70.00	9.80	46.16	27.80	17.94			131	9.78	7.8
Do	2	6	39.20	40.60	32.20	41.76	4.22	325	1,375	654	37.12	6.0
Actinomyces	None	26								30	2.5	7.8
Do	2	26						1,640	60	42	2.2	6.2
Bact. fluorescens.	None	7			32.56	41.40	25.40					8.1
Do	2	7			52.60	21.36	4.86	760	940			4.4

that this organism is capable of utilizing both the carbon and nitrogen of the particular amino acid.

Nawiasky (*18*) also found that *B. proteus* was capable of decomposing phenylalanine, with the liberation of 28.09 per cent of the nitrogen as ammonia, in 6 days. Among the products resulting from the decomposition of the phenylalanine, benzoic acid, phenylacetic acid, phenylpropionic acid, and phenyl-ethylamine were demonstrated, all benzol-ring compounds.

Blanchetière (*2*), however, found that *Bact. fluorescens liquefaciens* transformed only 6.4 per cent of the nitrogen of phenylalanine into ammonia, in 7 days, and 60.7 per cent in 43 days. Since no phenol or benzoic acid could be demonstrated in the medium, Blanchetière concluded that the organism is capable of breaking down the benzol ring.

DECOMPOSITION OF GLUTAMIC ACID

The chemical formula for glutamic acid is $HOOC.CH_2.CH_2.CH(NH_2)COOH$. This amino acid therefore contains 9.52 per cent nitrogen and 40.8 per cent of carbon, 4.28 being the ratio between the carbon and the nitrogen.

The content of amino acid actually found in the solution (Table IV) was lower than the theoretical amount. This is probably owing to the small amount of HCl present as an impurity. The Zygorhynchus decomposed 67 per cent of the amino acid, in the absence of dextrose, in 10 days, and the Trichoderma 63 per cent in 7 days; about half of the nitrogen was liberated as ammonia, about one quarter of the nitrogen was reassimilated by the organisms, and about a quarter of the nitrogen was present in solution in some different form. It has either been changed into another nitrogen compound, nonamino in nature, or it is a result of autolysis, which leads to a decrease in the nitrogen content of the mycelium and an increase of the organic and ammonia nitrogen in solution; the organic nitrogen secreted consists of various amino acids and hexone and purin bases, as shown by Reed (*21*) for Glomerella. It is probable that the discrepancy between the total nitrogen in solution and the sum of amino and ammonia nitrogen is due largely to the formation of other nitrogenous substances. This is brought out by the fact that discrepancies between the total nitrogen in solution and that accounted for by the residual amino acid and ammonia were often observed in the case of other amino acids. None seemed to show so large a difference as that found in the case of the glutamic acid.

The synthesis of mycelium was also much more extensive with this amino

TABLE IV.—*The chemistry of decomposition of 1 per cent glutamic acid by microorganisms, in the presence and absence of dextrose*

Organism used	Dextrose	Age of culture	Total N in 100 c. c.		NH_2—in 100 c. c.		NH_3—N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Decrease from control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None.		89.12		89.02		Tr.					3.0 [a](6.3)
Do	2		89.12		86.32		Tr.	1,815				3.0 [a](6.3)
Zygorhynchus	None.	10	75.96	13.16	29.44	59.58	25.4			204	13.35	5.7
Do	2	10	46.84	42.28	12.08	74.24	18.3	0	1,815	853	40.18	4.5
Trichoderma	None.	7	67.97	21.15	32.88	56.14	29.12			218	14.28	7.6
Do	2	7	58.75	30.37	47.26	39.06	5.48	460	1,355	545	29.12	3.4
Actinomyces	None.	13	70.80	18.32	6.28	82.74	28.36			169	13.44	9.5
Do	2	13	50.02	39.10	3.54	82.78	12.34	525	1,290	488	39.20	9.0
B. cereus	None.	15			64.68	24.34	5.16					8.8
Do	2	15			68.04	18.28	2.38	1,640	175			4.9
Bact. fluorescens	None.	5			15.43	73.59	28.50			128	12.02	9.1
Do	2	5			14.88	71.44	6.10	705	1,110	328	32.88	8.3

[a] Control for *Actinomyces*, *B. cereus*, and *Bact. fluorescens*.

acid than with the other three acids previously studied. This is attributable, on the one hand, to the much larger ratio of the carbon to the nitrogen in the particular amino acid, and, on the other, to the fact that glutamic acid is very favorable to respiration, resulting in the formation of very little volatile acid. Nawiasky (*18*) found that *B. proteus* will transform, in 3 days, 11.67 per cent of the nitrogen of glutamic acid to ammonia and, in 15 days, 52.97 per cent. The amount of ammonia, in comparison with the amount of mycelium synthesized is smaller in the case of the glutamic acid than in the case of glycocoll or alanine, as shown in the following summary:

Amino acid	C/N	Organism	Growth	NH_3—N	Growth / NH_3—N
			Mg.	*Mg.*	
Glycocoll	1.7	Trichoderma	50	24.28	2.0
Do	1.7	Actinomyces	59	30.46	2.0
Alanine	2.57	Zygorhynchus	48	6.40	7.5
Do	2.57	Trichoderma	80	21.98	3.6
Do	2.57	Actinomyces	126	39.17	3.2
Glutamic acid	4.28	Zygorhynchus	204	25.40	8.0
Do	4.28	Trichoderma	218	29.12	7.5
Do	4.28	Actinomyces	169	28.36	5.9
Do	4.28	B. fluorescens	128	28.50	4.5

The higher the ratio of carbon to nitrogen in the amino acid molecule, the less is the amount of ammonia produced per unit of protoplasm synthesized. This is based upon the simple principle that ammonia is a waste product in the carbon metabolism of an organism, with proteins or their derivatives as sources of energy; the amount of ammonia accumulated in the medium depends upon the growth of the organism and particularly upon the ratio of the available carbon to the available nitrogen in the medium; this holds true also when an available carbohydrate, like dextrose, is present in the medium; when the amount of available carbon is increased, the amount of ammonia accumulation, under the same conditions, will be decreased.

It is interesting to point in this connection to the work of Jodidi (*10*), who found that glycocoll, containing 18.7 per cent nitrogen, was decomposed in the soil and about 80 per cent of the nitrogen was transformed into ammonia, whereas leucine, with 10.7 per cent nitrogen, gave only 50 per cent of its nitrogen as ammonia. This is exactly what one might expect from the above considerations, since the C/N of glycocoll is 1.7 and the C/N of leucine is 5.1. The explanation given by Jodidi was that "the slower formation of ammonia from leucine is to be ascribed to the inert paraffin character of the comparatively long hydrocarbon chain, etc." Jodidi was correct, however, in respect to phenylalanine, which produced even less ammonia than the leucine, owing to the resistant character of its benzene ring.

Kelley (*11*) also brought out the fact that the ammonia content of the medium is definitely affected by its carbon content. The same amount of ammonia was formed from casein, soybean cake, cottonseed meal and linseed meal, when enough starch was added to each, so as to have the same amount of nitrogenous and nonnitrogenous substances, the carbon-nitrogen ratio actually affecting the ammonia formation in soils.

By comparing the ammonia formed by the three groups of organisms—the fungi, actinomycetes, and bacteria—an increasing amount of ammonia is found to be formed per unit of protoplasm synthesized, in order named. This holds true in spite of the fact that fungi contain the smallest percentage of nitrogen, and can be explained only by a proper understanding of the metabolism of these three groups of organisms. The fungi reassimilate the largest proportion of carbon; therefore they will also reassimilate a proportionately larger amount of nitrogen and leave a proportionately smaller amount of waste nitrogen as ammonia.

The actinomycetes, and especially the bacteria, which produce a smaller amount of growth, use the available energy less economically; they assimilate less of the nitrogen, waste more of the carbon (as CO_2), and therefore liberate a larger amount of ammonia, as shown in the summary taken from Table IV (shown on page 273).

For approximately the same amount of ammonia formed, the fungi decomposed less amino acid and produced a heavier growth than the Actinomyces and Bacterium.

SUMMARY

Organism	Amino acid decomposed	Growth	NH_3—N
	Mg.	*Mg.*	*Mg.*
Zygorhynchus	59. 58	204	13. 65
Trichoderma	56. 14	218	14. 28
Actinomyces	82. 74	169	13. 44
Bact. fluorescens	73. 59	128	12. 02

In the presence of dextrose in the medium, the same process took place as with the other amino acids: A larger amount of protoplasm was synthesized; a larger amount of nitrogen was reassimilated, and a smaller amount of ammonia was left in the medium. The Actinomyces is again an exception; it continued to decompose the amino acid with the liberation of ammonia,

TABLE V.—*The chemistry of decomposition of 1.4 per cent glutamic acid by microorganisms, in the presence and absence of dextrose*

Organism used	Dextrose	Age of culture	Total N in 100 c. c.		NH_2—N in 100 c. c.		NH_3—N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Decrease from control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None.		126. 40		126. 40							7. 3
Do	2		126. 40		126. 40			1, 800				6. 4
Zygorhynchus	None.	15			122. 24	4. 16	3. 62			30	2. 6	8. 4
Do	2	15			104. 96	21. 44	4. 12	65	1, 735	473	17. 92	7. 1
Trichoderma	None.	13			122. 60	3. 80	1. 76			28	2. 0	8. 2
Do	2	13			94. 64	31. 76	3. 00	155	1, 645	627	30. 49	6. 9
Actinomyces	None.	19	108. 08	18. 32	61. 46	64. 96	34. 04			141	11. 60	9. 8
Do	2	19	100. 16	26. 24	89. 49	36. 91	9. 18	1, 080	720	350	27. 20	8. 7
B. cereus	None.	7			113. 78	12. 62	0. 52					7. 6
Do	2	7			117. 04	9. 36	1. 76	1, 710	90			5. 8
Bact. fluorescens	None.	7	86. 03	40. 37	28. 91	97. 49	22. 89			312	32. 32	9. 4
Do	2	7	48. 35	78. 05	13. 68	112. 62	9. 36	525	1, 275	831	78. 48	8. 9

TABLE VI.—*The chemistry of decomposition of 1 per cent asparagine by microorganisms, in the presence and absence of dextrose*

Organism used	Dextrose	Age of culture	Total N in 100 c. c.		NH_2—N in 100 c. c.		NH_3—N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Decrease from control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None.		179. 5		90. 9		Trace.					7. 0
Do	2		179. 5		93. 0		Trace.	1, 840				6. 4
Zygorhynchus	None.	6	177. 0	2. 5			50. 40			21	1. 6	8. 0
Do	None.	9	174. 0	5. 5	73. 4	17. 5	69. 56			39	3. 04	8. 6
Do	2	6	142. 0	37. 5	71. 5	21. 5	31. 30	60	1, 780	321	25. 5	7. 0
Do	2	9	155. 0	24. 5	56. 3	36. 7	83. 56	0	1, 840	223	13. 44	7. 7
Trichoderma	None.	4	174. 0	5. 5	80. 8	10. 1	8. 31			18		7. 5
Do	None.	12	174. 0	5. 5	84. 7	6. 2	74. 66			16		8. 6
Do	2	4	137. 0	42. 5	67. 2	25. 8	3. 81	670	1, 170	643	39. 86	6. 4
Do	2	12	134. 0	45. 5	48. 3	44. 7	65. 12	0	1, 840	502	36. 20	7. 9
Actinomyces	None.	22	169. 1	10. 4	77. 2	13. 7	18. 48			41	2. 83	7. 7
Do	2	9	159. 5	20. 0	78. 9	14. 1	5. 76	1, 070	770	176. 5	17. 87	6. 3
Do	2	22	148. 4	31. 1	19. 6	73. 4	65. 76	0	1, 840	249	22. 58	8. 4
Do	2	22	164. 4	15. 1	71. 6	21. 4	4. 48	940	900	167	14. 57	6. 0
B. cereus	None.	13	150. 0	29. 5	56. 2	34. 7	55. 54			17		8. 6
Do	2	13	174. 0	5. 5	72. 0	20. 8	30. 64	1, 560	280			6. 2
Bact. fluorescens	None.	3	156. 0	23. 5	69. 9	21. 0	86. 80			53	6. 17	8. 6
Do	None.	7	113. 0	66. 5	28. 5	62. 4	91. 90					8. 6
Do	2	3	151. 0	28. 5	66. 2	26. 8	78. 50	1, 340	500	322	28. 64	8. 0
Do	2	7	108. 0	71. 5	37. 0	56. 0	64. 50	50	1, 790	Lost.		7. 8

showing that this organism will also prefer glutamic acid as a source of energy, even in the presence of dextrose.

Table V contains only some confirmatory data. Owing to the alkaline reaction, the fungi were soon injured in their development, in the absence of dextrose, by the rapidly accumulating ammonia. The Actinomyces and the bacteria, however, that can stand a higher alkalinity than the fungi, decomposed a great deal of the glutamic acid, even in the 1.4 per cent concentration, with results similar to those obtained in the previous experiment.

DECOMPOSITION OF ASPARAGINE

The chemical formula for asparagine is $NH_2.CO.CH_2.CH(NH_2)COOH(+H_2O)$; it contains 18.68 per cent nitrogen and 32 per cent carbon, the ratio of carbon to nitrogen being 1.7, very similar to glycocoll. However, the two NH_2 groups in the asparagine molecule are in different positions, one as an amino and the other as an amide group. The various references in the literature on the decomposition of asparagine tend to indicate that the splitting off of these two groups and their transformation into ammonia take place with unequal rapidity.

The asparagine is first changed to aspartic acid and ammonia, then to succinic acid; the aspartic acid may also change to acetic acid:

$$NH_2.CO.CH_2.CH(NH_2)COOH+H_2O=HOOC.CH_2.CH(NH_2)COOH+NH_3$$

$$HOOC.CH_2.CH(NH_2)COOH\ (CH_2COOH)_2+NH_3$$

$$(CH_2COOH)_2+70=4\ CO_2+3H_2O$$

Nawiasky (*18*) found that *B. proteus* reduces, in 24 hours, almost all the asparagine to aspartic acid and ammonia. The aspartic acid changes, at the same time, to succinic or acetic acids and ammonia.

In our own experiments (Table VI), Zygorhynchus decomposed, in 9 days, in the absence of dextrose, 17.5 mg. amino nitrogen out of a total of 90.9 mg.; however, there were 69.56 mg. of ammonia nitrogen in the medium, showing that the largest amount of ammonia originates from the amide group. The amount of growth produced by both fungi is very small, in comparison with the amount of ammonia formed and is more comparable with the decrease in the amino acid content. This points to two things: (1) The amide group is split off more readily than the amino group (the latter only as far as the aspartic acid is decomposed as a source of energy); (2) the rapid change in reaction to alkalinity, due to the accumulation of ammonia, represses the further development of the fungi and the amount of growth as well as amino acid decomposed is limited. The amount of available carbon in the asparagine molecule is so small, in comparison with the available nitrogen, that only a small amount of growth may be formed. The Actinomyces produced 41 mg. of growth, with a comparatively small amount of amino nitrogen decomposed and ammonia formed, in the absence of dextrose. The largest amount of ammonia was produced by the *Bact. fluorescens*: 86.80 mg. of ammonia nitrogen was found in the medium after 3 days, while only 21.0 mg. of amino nitrogen had been decomposed. The fact that large amounts of ammonia are formed in the medium even in the presence of dextrose indicates that, although ammonia formation from the amino acid is a direct result of metabolism, the formation of ammonia from an amide may be primarily a result of enzyme action, regardless of whether the ammonia is needed by the organism. This is further substantiated by the fact that the amount of ammonia rapidly increases with age of culture, independent of the actual carbon assimilation.

The addition of dextrose tends to keep down the amount of ammonia accumulated in the medium, especially in the case of the fungi as a result of the probable reassimilation of the ammonia as long as there is available energy. However, as soon as the energy is used up, the ammonia begins to accumulate rapidly. This is clearly seen in the case of the Actinomyces, for example. After 9 days' incubation, only 770 mg. of dextrose was decomposed, 176.5 mg. of mycelium was resynthesized, and only 5.76 mg. ammonia were found in the medium. After 22 days, the duplicate cultures showed different results; one made further growth and the other did not develop any further than at the first period of analysis. It is interesting to note that the culture containing some undecomposed dextrose, after 22 days, showed about the same relationship in growth, reaction, decomposition of amino nitrogen and ammonia formation as the 9-day-old culture. In the second flask, however, where all the dextrose had disappeared, the decomposition of the amino acid advanced rapidly, accompanied by ammonia accumulation and change in reaction. The Actinomyces seemed to

have produced all or most of its ammonia from the amino acid group and not from the amide group, while the *Bact. fluorescens* seemed to have produced most of its ammonia from the amide and not from the amino acid group. This again goes to emphasize that the decomposition of proteins and amino acids and accumulation of ammonia are results of the metabolism of the particular microorganisms, under specific conditions. The changes produced as a result of decomposition of the protein and amino acid molecules do not depend so much upon the age of the culture as upon its development; that is, a more abundant growth in a shorter period of time will produce a series of changes parallel to its growth rather than its age.

The *B. cereus* made only a very limited growth with asparagine; in the rapidity of deamidization it behaved similarly to the *Bact. fluorescens*.

CHEMISTRY OF DECOMPOSITION OF CASEIN

Casein is soluble in dilute alkali solutions and can therefore be readily used as a source of nitrogen and carbon for the activities of microorganisms. Only a small amount of the casein nitrogen is present in the lysin group, which accounts for the small amount of amino nitrogen present in the control solution. The two fungi and Actinomyces readily attacked the casein; the two bacteria behaved, however, in an exactly reverse manner than they did in respect to the amino acids. The *B. cereus* was very active and *Bact. fluorescens* hardly attacked the casein at all.

The Zygorhynchus is capable of decomposing casein, but very little amino nitrogen accumulates in the medium, showing that it decomposes the amino acids as soon as they are formed. One would almost expect this to occur, since this organism was capable of utilizing, as a source of carbon and nitrogen, all the amino acids previously tested, if only present in the proper concentration and with the proper reaction. The casein as such was all decomposed, since no precipitate was obtained any longer with acetic acid. It was not, however, completely decomposed, even in the presence of dextrose, to carbon dioxide, water, and ammonia, as seen from the fact that, although 120.5 mg. of the nitrogen was present in solution, only 17 mg. of it was found in the form of amino and ammonia nitrogen. In other words, a large part of the nitrogen was left in the form of polypeptides and other protein derivatives, which the organism could not very readily and rapidly utilize as a source of carbon. In the presence of available dextrose, a large part of the

TABLE VII.—*The chemistry of decomposition of 1 per cent casein by microorganisms, in the presence and absence of dextrose*

Organism used	Dextrose	Age of culture	Total N in 100 c. c.		NH_3—N in 100 c. c.		NH_3—N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Increase over control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None.		126.4		6.84		2.10					7.0
Do	2		126.4		6.84		2.10	1,890				6.4
Zygorhynchus	None.	16	120.5	5.9	10.32	3.48	13.52			93	6.35	7.8
Do	2	16	76.1	50.3	9.48	2.64	19.82	0	1,890	643	40.95	7.8
Trichoderma	None.	6	113.1	13.3	26.24	19.40	14.14			154	14.99	7.5
Do	2	6	70.7?	55.7?	15.76	8.92	3.00	560	1,330	452	19.90	4.6
Do [a]	None.	5			26.17		3.31			59		7.2
Do [a]	None.	11			40.02		24.29			200	19.62	8.2
Do [a]	2	5			16.94		1.66	[b]1,385	335	92		5.5
Do [a]	2	11			21.90		9.11	[b]40	1,680	692	44.90	6.6
Actinomyces	None.	19	108.9	17.5	8.94	2.10	31.80			117	9.95	8.0
Do	2	19	104.2	22.2	15.40	8.36	3.00	1,380	510			5.1
B. cereus	None.	10	101.1	25.3	18.10	11.26	45.50			102	8.07	8.4
Do	2	10	120.0	6.4	9.75	2.91	3.30	1,700	190			4.8
Do [a]	None.	6			54.04		24.92			66		8.5
Do [a]	None.	13			24.77		38.52			156		8.6
Do [a]	2	6			25.91		2.8	[b]1,490	230			4.7
Bact. fluorescens.	None.	13	124.3	2.1	8.34	1.50	2.36					7.1
Do	2	13	105.8	20.6	7.78	0.94	1.12	1,580	310			4.6

[a] Data from a different experiment.

[b] Control in this case 1,720 mgm.

nitrogen was reassimilated by the organism and, after all the dextrose was used up, ammonia began to accumulate in the medium; only a small part of the nitrogen was present in this case in the form of polypeptides.

Similar results were obtained with the Trichoderma. In the absence of dextrose, the organism produced a more abundant growth than the Zygorhynchus, and it also changed about 40 per cent of the nitrogen in solution to amino nitrogen and ammonia. The fact that Trichoderma is capable of breaking down proteins more vigorously than Zygorhynchus is further indicated by repeated experiments on casein decomposition: After 11 days, there was present in the medium 40.02 mg. of amino nitrogen and 24.29 mg. of ammonia nitrogen, or nearly 60 per cent of the total nitrogen. Dextrose exerts a protective action, both on the hydrolysis of the protein and upon ammonia formation, similar to the phenomena elucidated above. As long as there is dextrose left in the medium, the reaction will be acid (causing a precipitation of the casein, followed by its dissolution as soon as the dextrose is used up), and very little ammonia will accumulate.

The Actinomyces behaved in as imilar way. There was, however, an increase in the amino nitrogen content in the dextrose containing media; the ammonia accumulated largely in the dextrose-free media.

B. cereus attacked casein more vigorously in the absence of dextrose than in its presence. There was at first a rapid increase in the amino nitrogen content in the absence of dextrose followed by a decrease, probably owing to the fact that, after some time, the increase in the amino nitrogen content resulting from the hydrolysis of the protein, then of the protein derivatives, was balanced and finally exceeded by the increase in the ammonia resulting from the decomposition of the amino compounds. It is interesting to note that there was a decided increase in the amino nitrogen content even in the presence of dextrose, confirming the observations of DeBord (*5*), although in this case it was lower than in the absence of dextrose. The ammonia accumulation was insignificant in the presence of dextrose. This would indicate that, although the protein molecule may be hydrolyzed by *B. cereus* even in the presence of dextrose, the hydrolysis does not go much further than the amino acid stage, so that no ammonia can accumulate. In view of the fact that *B. cereus* does not attack readily simple amino acids, at least not those that were tested, one would expect that the hydrolysis of casein by *B. cereus* would lead to an accumulation of certain amino acids; since ammonia results chiefly when the amino acids are used as sources of energy (comparatively little energy being liberated in the hydrolysis of the protein to the amino acids), no ammonia would accumulate in the presence of an available carbohydrate. In the absence of dextrose, the organism has to utilize the amino acids as sources of energy, and the results seem to indicate that *B. cereus* is capable of decomposing at least certain fractions of the protein molecule, when ammonia is formed and allowed to accumulate in the medium.

Bact. fluorescens was found unable to utilize casein, either as a source of carbon or of nitrogen; the fact that small amounts of dextrose were used would lead one to think there were certain substances present in the casein which were utilized by the organisms as sources of nitrogen.

The two bacteria behaved thus in a distinctly different manner toward a protein and its derivatives: *B. cereus* is typically proteolytic, capable of decomposing native proteins (the same was found to hold true in the case of purified vegetable proteins, as will be shown elsewhere), while *Bact. fluorescens* is nonproteolytic but can utilize amino acids (and probably also polypeptides) as sources of energy and nitrogen. By combining the two organisms, one would logically expect that the course of transformation of the protein molecule would take place in a distinctly different manner than by either organism alone. This was actually found to be the case, as shown in Table VIII and Figure 1.

B. cereus alone hydrolyzed the casein with a gradual accumulation of amino nitrogen and ammonia, so that, in 6 days, 35 per cent of the nitrogen in solution was in the form of amino nitrogen and 12 per cent in the form of ammonia. After that the total content of amino nitrogen decreased, owing to its transformation to ammonia, so that, in 15 days, the total amino nitrogen dropped from 44.89 mg. (6 days) to 24.62 mg. (about 30 per cent of the nitrogen in solution); the ammonia rose in that period of time from 16.62 mg. to 46.52 mg., or from 12.5 per cent to over 50 per cent of the nitrogen in solution. This indicates definitely that the hydrolysis of the polypeptides continues even after the maximum amino nitrogen has been reached; only more of it is changed to ammonia.

When *Bact. fluorescens* and *B. cereus* were inoculated into the same solution, the latter decomposed the casein, while the former decomposed a large part of the simple amino compounds, as soon as formed, with the formation of ammonia. The number of cells of *Bact. fluorescens* greatly exceeded those of *B. cereus;* the culture looked and smelled like a typical *Bact. fluorescens* culture. The accumulation of amino nitrogen in the mixed culture never reached as high a point as in the culture of *B. cereus* alone; so that, in 6 days, the mixed culture produced a little more than half as much amino nitrogen, as *B. cereus* alone did, and over twice as much ammonia.

The question often arises in the study of soil microbiology: Just what part is played in the soil by a large number of bacteria and other organisms incapable of decomposing native proteins, celluloses, and other complex compounds added to the soil? It may be that here will be found the answer to this question; namely, that they attack the products resulting from the decomposition of complex compounds by those organisms that are able to do so. Various instances of such associative action are on record as between the nitrate-forming and nitrite-forming bacteria, nitrogen-fixing and cellulose-decomposing bacteria, etc. There is no reason to doubt that a similar associative action is found here between the large spore-forming and other strongly proteolytic bacteria, (aerobic and anaerobic) and fungi, on the one hand, and the small, nonspore forming and nonproteolytic bacteria on the other. This will also explain the apparent discrepancy between the numbers of the spore-forming and nonspore-forming bacteria in the soil, the former being present there only as 5 to 10 per cent of the flora developing on the plate, and the latter as 50 to 80 per cent. This discrepancy led Conn (*4*) to doubt the activity of the spore formers in the soil. The explanation here may again be very simple: The strong proteolytic cells will obtain a comparatively small amount of energy from the hydrolysis of the proteins to amino acids, even when they transform a part of these to ammonia; this and the large size of the cell may account for the fact that only a limited number of cells are capable of

TABLE VIII.—*Decomposition of casein by B. cereus, by Bact. fluorescens and by both organisms in association*

Age of culture	Organism used: B. cereus					Bact. fluorescens			
	Total N in 100 c. c. solution	NH_2—N in 100 c. c.	NH_3—N in 100 c. c.	Weight of pellicle	pH	Total N in 100 c. c. solution	NH_2—N in 100 c. c.	NH_3—N in 100 c. c.	pH
	Mg.	*Mg.*	*Mg.*	*Mg.*		*Mg.*	*Mg.*	*Mg.*	
Control	140.6	7.57	2.30	----------	7.1	140.6	7.57	----------	7.1
1 day	----------	9.55	3.30	----------	7.1	----------	6.34	3.00	7.1
2 days	----------	15.30	3.00	----------	7.1	----------	9.86	1.04	7.1
4 days	135.03	29.93	12.94	50	7.4	----------	7.90	1.40	7.1
6 days	128.1	44.89	16.62	73	8.0	----------	8.21	2.06	7.3
9 days	113.2	38.93	40.86	113.2	8.5	----------	7.46	3.00	7.3
15 days	82.56	24.62	46.52	209	8.9	139.6	9.86	0.88	7.3

Age of culture	Organism used—B. cereus+Bact. fluorescens				
	Total N in 100 c. c. solution	NH_2—N in 100 c. c.	NH_3—N in 100 c. c.	Weight of pellicle [a]	pH
	Mg.	*Mg.*	*Mg.*	*Mg.*	
Control	140.6	7.57	2.30	----------	7.1
1 day	----------	7.26	1.76	----------	7.1
2 days	----------	18.42	7.96	----------	7.2
4 days	119.80	23.31	27.86	156	8.2
6 days	115.50	27.68	35.36	185	8.9
9 days	92.14	13.90	56.76	203	9.4
15 days	91.22	10.84	59.72	224	9.4

[a] Approximate.

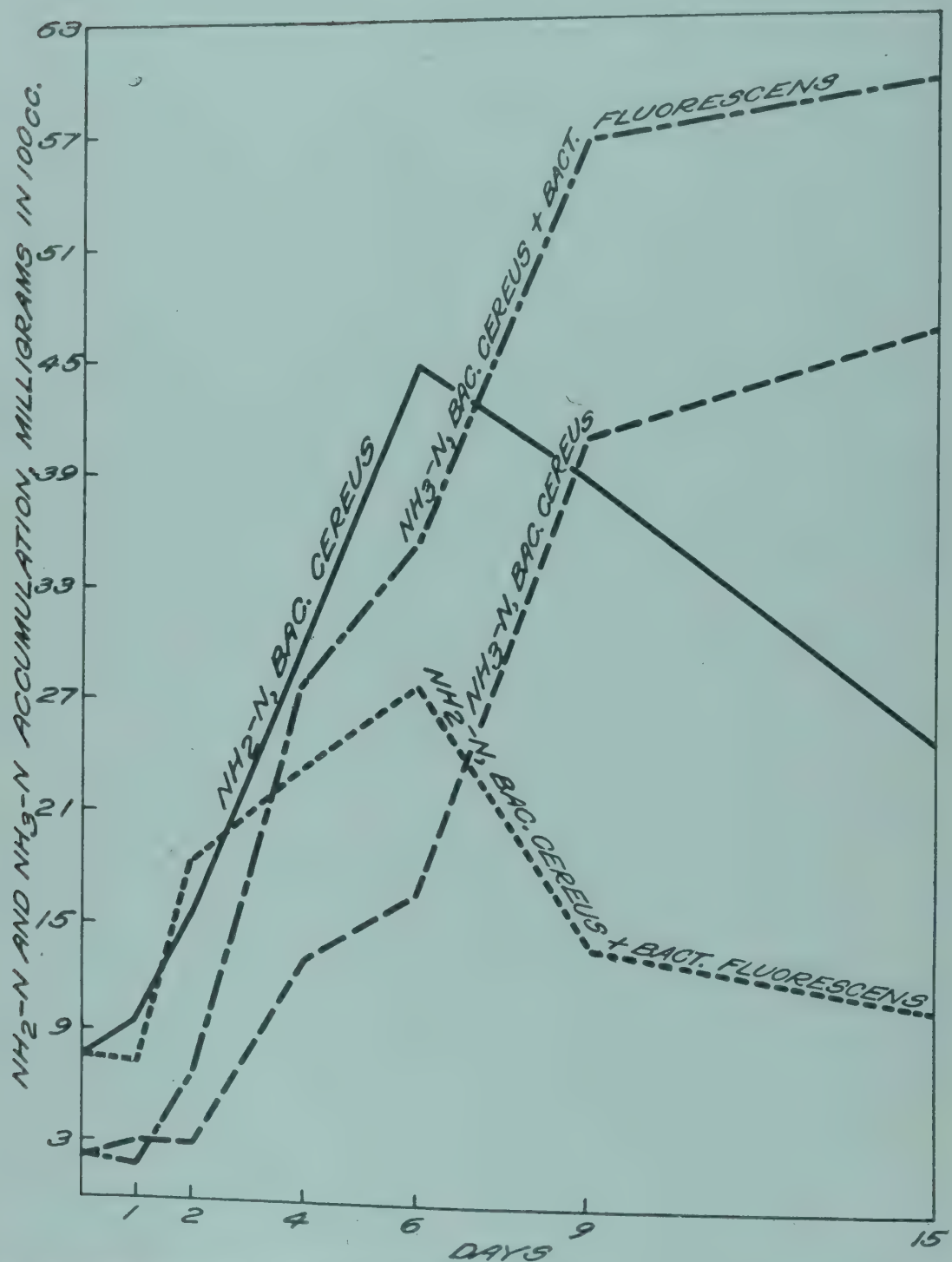

FIG. 1.—Course of formation and accumulation of NH_2—N and NH_3—N in casein solution by *Bac. cereus* and *Bac. cereus*+*Bact. fluorescens*

developing with the amount of energy made available. The small nonspore-forming bacteria, utilizing protein derivatives and breaking these down to ammonia, will obtain a comparatively larger amount of energy; this fact, as well as the smaller size of the cell, may be the cause for the development of a large number of individuals.

It remains to be seen whether this holds true also for other bacteria, or the two bacteria used in this study just happen to be exceptions.

One more point remains to be discussed in this preliminary study on the decomposition of proteins and their derivatives by microorganisms; namely, the influence of carbohydrates upon the decomposition of the proteins and upon ammonia accumulation. It was pointed out above that the theory that "available carbohydrates exert a protective action upon the decomposition of proteins by microorganisms" should be modified, since, as seen in the case of the Actinomyces, some organisms are capable of utilizing proteins as sources of energy, even in the presence of dextrose, probably the most available of all carbohydrates for microorganisms, including actinomycetes. Table IX contains a few data on the influence of other carbohydrates upon the decomposition of casein by bacteria and fungi. Since *B. cereus* is unable to decompose celluloses, a strong cellulose-decomposing bacterium *Bact. fimi* of Kellerman and associates, was used. In the case of *B. cereus*, both cane sugar and starch exerted a protective action upon ammonia accumulation, starch to a lesser extent, possibly because it is not so readily available as the sugars. In the case of Trichoderma, cane sugar and starch also exerted a protective action, while cellulose did not. Trichoderma, one of the strongest cellulose-decomposing organisms ever tested in this laboratory, behaves towards the protein as if the cellulose were not present in the medium at all; in other words, it prefers the protein as a source of energy to the available carbohydrate (cellulose). The *Bact. fimi*, however, decomposed the protein only to a very limited extent and preferred the carbohydrate as a source of energy.

The above theory should therefore be modified to a simple problem of metabolism, which holds true not only for microorganisms but for all living beings that depend for their energy upon complex substances; namely, that a living being derives its energy from a substance, which is most available to it and which may be specific for a particular organism; a large number of microorganisms prefer certain simple available carbohydrates to proteins as sources of energy, and in the presence of those, the proteins will be utilized only as sources of nitrogen; therefore, ammonia, which is chiefly a product of utilization of proteins as sources of energy, will not accumulate.

SUMMARY

A study has been made of the nature of decomposition by two fungi, two bacteria, and one Actinomyces of certain pure amino acids and casein; the latter was used in a synthetic medium as sources of nitrogen alone and of

TABLE IX.—*Influence of various carbohydrates (2 per cent) upon the decomposition of casein by microorganisms* [a]

Carbohydrate	Organism									
	B. cereus			Trichoderma				Bact. fimi		
	NH_2—N in 100 c. c.	NH_3—N in 100 c. c.	pH	NH_3—N in 100 c. c.	NH_3—N in 100 c. c.	Weight of dry mycelium	pH	NH_2—N in 100 c. c.	NH_3—N in 100 c. c.	pH
	Mg.	*Mg.*		*Mg.*	*Mg.*	*Mg.*		*Mg.*	*Mg.*	
Control	8.4	1.76	7.7	8.4	1.76	-------	7.7	8.4	1.76	7.7
None	40.28	32.96	8.2	40.70	19.77	94	8.1	---------	---------	------
Cane sugar	27.54	3.00	4.7	47.17	10.74	173	8.0	---------	---------	------
Starch	28.33	8.86	5.1	20.17	9.82	643	7.1	---------	---------	------
Cellulose	---------	---------	------	[b] 30.32	34.06	96	8.2	[c] 12.88	1.12	6.2

[a] Incubation 8 days; for cellulose flasks, 16 days.
[b] No cellulose decomposed.
[c] 65 mg. of cellulose decomposed.

nitrogen and carbon. The reactions taking place were followed by measuring the residual amino nitrogen formed from decomposition of casein, formation and accumulation of ammonia, amount of growth produced by the organisms, and disappearance of dextrose in the medium, wherever it has been used.

The results indicate that not all organisms attack proteins and amino acids alike. The two fungi *Trichoderma koningi* and *Zygorhynchus molleri* utilized the various amino acids and the protein both as sources of carbon and nitrogen, the amount of growth and ammonia accumulation depending, however, in the absence of available carbohydrates, upon the available carbon in the amino acid molecule. A definite relation was found to exist between the carbon (available) content of the amino acid molecule and the amount of ammonia accumulating. The two bacteria tested, *B. cereus* and *Bact. fluorescens*, behaved differently; the first was unable to attack glycocoll, alanine, and phenylalanine, while glutamic acid and asparagine were acted upon to a limited extent, and casein and other native proteins were decomposed very rapidly. The *Bact. fluorescens* was unable to decompose casein, but acted upon the various amino acids used very readily. By combining the two organisms in casein media, the protein was decomposed very rapidly to ammonia, the *B. cereus* hydrolyzing the casein chiefly to protein derivatives and the *Bact. fluorescens* decomposing the latter to ammonia.

Ammonia accumulation can not be used as an index of the proteolytic activities of microorganisms, when the carbon content of the medium is not considered; an organism may decompose a much larger amount of protein in the presence of an available carbohydrate, but produce a much smaller amount of ammonia.

The Actinomyces was found to be capable of utilizing amino acids and proteins as sources of energy, thus allowing an accumulation of ammonia, even in the presence of dextrose.

Ammonia formation by microorganisms from amino acids depends upon the carbon-nitrogen ratio of the compound, as well as upon the nature of the organism, as influenced by its utilization of energy.

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A BIOMETRIC COMPARISON OF THE UREDINIOSPORES OF CRONARTIUM RIBICOLA AND CRONARTIUM OCCIDENTALE[1]

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INTRODUCTION

It appears to be impracticable to differentiate between *Cronartium ribicola* Fischer and *Cronartium occidentale* Hedgcock, Bethel, and Hunt in the uredinial stage by any ordinary means of visual inspection or low power microscopical examination. Both rusts attack a large number of species of the genus Ribes. Superficially and structurally the uredinia resemble each other very closely; and the urediniospores of the two species differ only slightly—so slightly in fact that the differences escape observation unless special technique is used. The fact that *C. ribicola* is already present in the northwestern United States makes the problem of differentiation something more than a matter of academic interest, on account of the necessity of recognizing advance infections on Ribes.

Hedgcock, Bethel, and Hunt[3] give the size of the urediniospores of *C. occidentale* as 18.5 to 32 by 13.5 to 20μ averaging 24 by 16μ, and state that the wall is 2 to 3μ thick. Colley[4] has described the size of the urediniospores of *C. ribicola* roughly as 19 to 45 by 10 to 20μ. These two range descriptions are not comparable, because the measurements on which the figures are based were not made by similar methods. Granting that they are comparable, however, the range descriptions would not be a sufficiently sound basis for diagnosis, for there is no means of knowing the distribution of the more common spore sizes within the limits of the ranges. Of the many hundreds of spore measurements made on the urediniospores of the two species within the last few years, some have been made on fresh spores and some on dry spores, with and without the use of special mounting media. Obviously these results also are more or less unsatisfactory. The object of this paper is the presentation of an analysis of strictly comparable measurements made on 3,000 urediniospores of each species.

METHODS

SELECTION OF MATERIAL

Herbarium material was selected for the measurement study. Experience had shown clearly that measurements made on fresh spores were not comparable with measurements made on dry spores; and it was obvious that herbarium specimens had one thing at least in common—they were all dry. Furthermore, it was possible to select specimens from the herbarium covering the widest possible range for host, locality, and time of collection. The spores were taken from specimens which appeared to be clean and well preserved, and from sori which appeared to be mature.

The selected specimens were grouped in three series. In each series there were 10 sets of spores—each set consisting of 100 urediniospores of *Cronartium ribicola* and 100 urediniospores of *Cronartium occidentale*. The description of the three series follows:

Field series.—A selection of 50 specimens of each species from collections made in the field; 5 specimens from each species in a set; set numbers 1–10, inclusive.

Block Island series.—A selection of 10 specimens of each species from collections made in experimental plots located on Block Island, off the coast of Rhode Island; one specimen of each species in each set; set numbers 11–20, inclusive.

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[4] COLLEY, R. H. PARASITISM, MORPHOLOGY, AND CYTOLOGY OF CRONARTIUM RIBICOLA. Jour. Agr. Research 15: 619–660, illus. 1918.

Greenhouse series.—A selection of 10 specimens of each species from collections made in the pathological greenhouses at Washington, D. C., one specimen of each species in each set; set numbers 21-30, inclusive.

placed in a small drop of the following medium:[5]

Potassium acetate	10 gms.
Distilled water	500 cc.
Pure glycerine	200 cc.
Ethyl alcohol, 95 per cent	300 cc.
Erythrosin	10 gms.

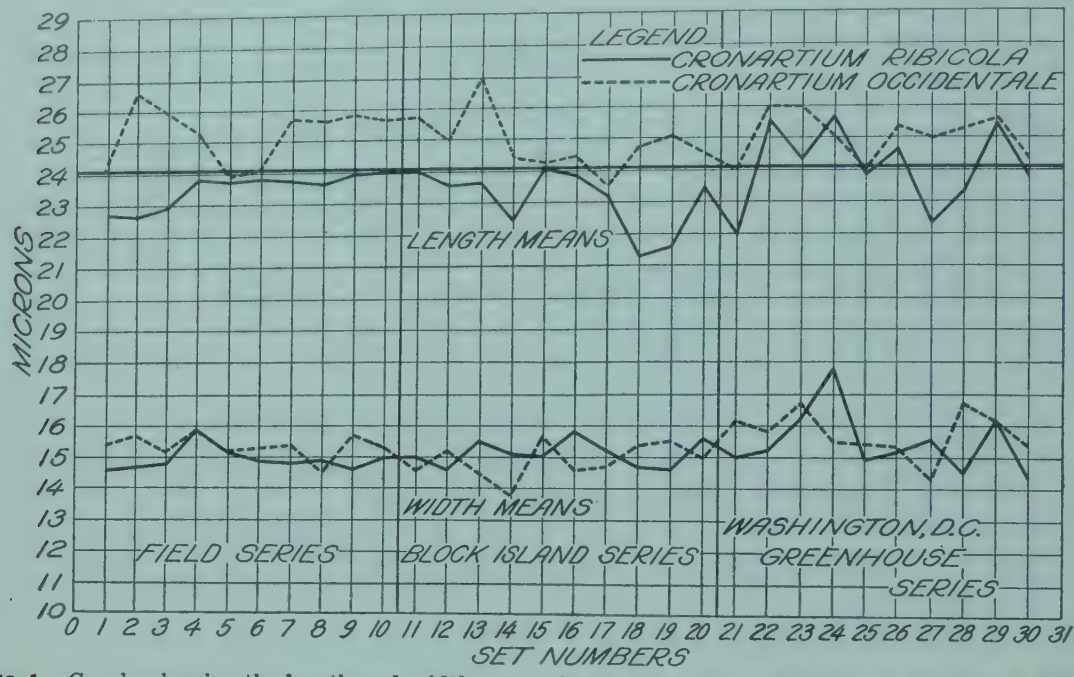

FIG. 1.—Graphs showing the length and width means for 30 sets of urediniospores of *Cronartium ribicola* and *Cronartium occidentale*. Each point on each species line represents the mean of 100 measurements. The series are explained in the text. The set numbers correspond to those in the last columns of Tables I to IV

The collection data are given in full in Tables I to IV.

MOUNTING

The spores were removed from the uredinia as carefully as possible and

They were left in this medium without being covered with a cover glass for several hours. The mount was then finished by adding a drop of glycerine jelly made up as follows:

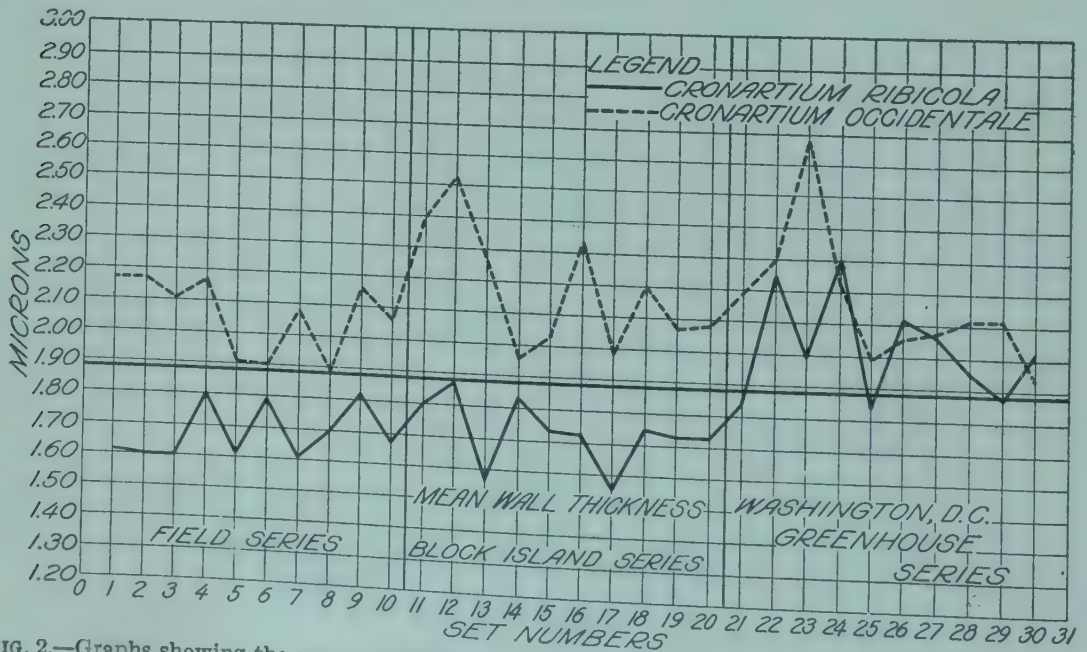

FIG. 2.—Graphs showing the mean wall thickness for 30 sets of urediniospores of *Cronartium ribicola* and *Cronartium occidentale*. Each point on each species line represents the mean of 50 measurements. Series and set numbers as in Figure 1

[5] This medium has been used for years in the Bureau of Plant Industry by Dr C. L. Shear and others. The only modification of their formula is the addition of the erythrosin.

Distilled water	42 cc.
Pure glycerine	50 cc.
Gelatine	7 gms.
Phenol	1 cc.
(Use 1 cc. fresh acid or 1 gm. of crystals.)	
Erythrosin	1 gm. in 9 cc. distilled water

The formula is based on one given by Moreau.[6] The erythrosin should be dissolved as far as possible in the 9 cc. of distilled water and the solution then added to the glycerine gelatine mixture *after* it has been cooked but *before* it has cooled.

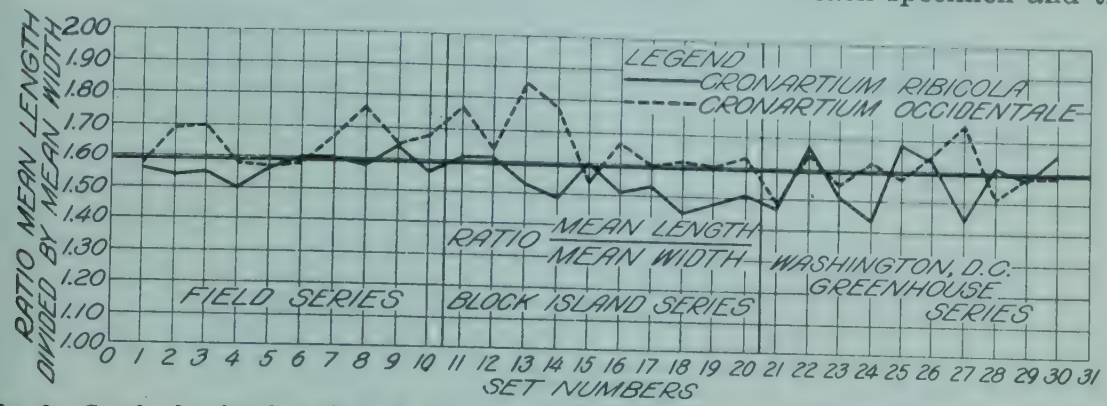

FIG. 3.—Graphs showing the ratio for the mean length divided by the mean width for 30 sets of urediniospores of *Cronartium ribicola* and *Cronartium occidentale*. Series and set numbers as in Figure 1

MEASURING

All the spores were measured by projection. The apparatus[7] was so arranged that the images of the spores were projected at a magnification of 1,000 diameters on a white field. The images of such spores as fell within a 4-inch circle in this white field were then measured to the nearest millimeter with a standard white-faced millimeter scale. Wall thickness was measured to the nearest half millimeter. The mount was moved across the field of vision systematically by means of a mechanical stage.

In the *Field series* 20 spores were measured from each specimen and the measurements from each 5 specimens grouped at random into sets, as shown in Table I and Table II; but in the *Block Island series* and the *greenhouse series* 100 spores were measured from each specimen. Wall measurements were made on 50 of each 100 spores measured. For each series, therefore, there were 1,000 length measurements, 1,000 width measurements, and 500 wall measurements.

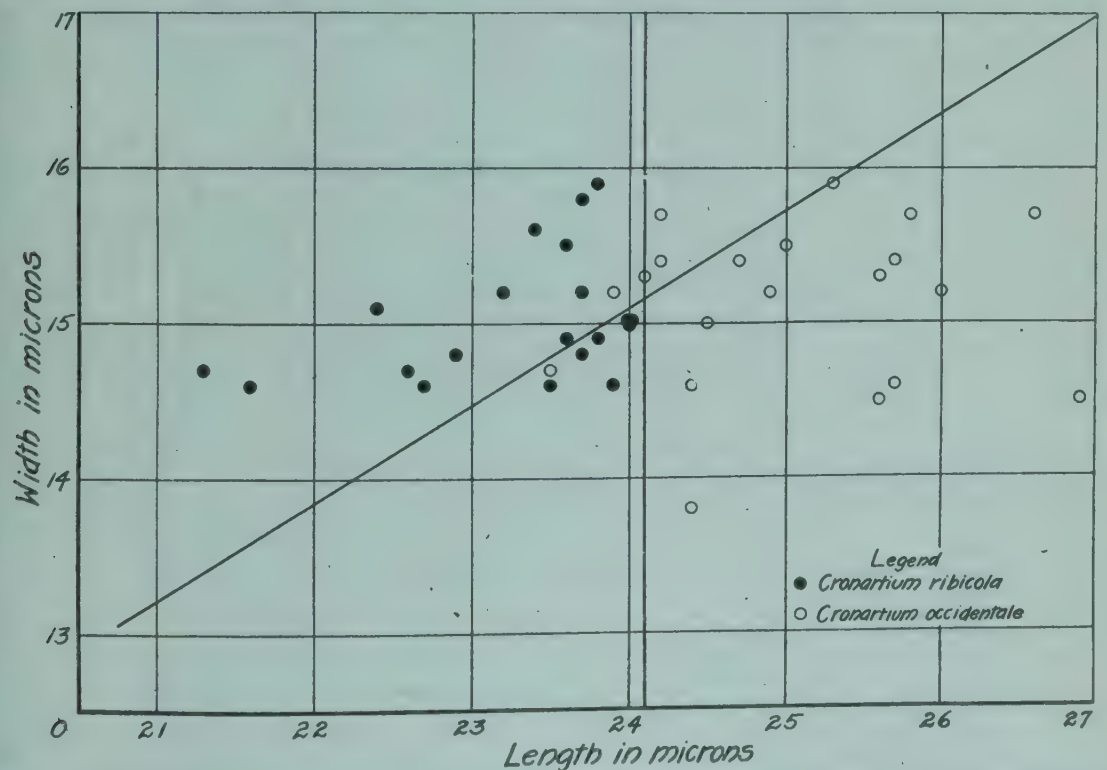

FIG. 4.—Graphic representation of the application of the criteria of size and shape of urediniospores to the biometric diagnosis of *Cronartium ribicola* and *Cronartium occidentale*. Each plotted point represents the length and width mean of 100 measurements. The data are taken from the figures given in Tables I–III for sets 1–20. Further explanation in the text

[6] MOREAU, F. NOTIONS DE TECHNIQUE MICROSCOPIQUE.—APPLICATION Á L'ÉTUDE DES CHAMPIGNONS. Bul. Trimest. Soc. Mycol. France 34; 137–191, illus. 1918.

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ANALYSIS OF THE MEASUREMENT RESULTS

The measurements were tallied and analyzed by ordinary statistical methods. The means for the length and width and wall thickness and the ratio of mean length divided by mean width for each of the 30 sets of spores are given in Tables I to IV. A summary of the measurement data is given in Table V.

The data in Tables I to IV are presented graphically in Figures 1, 2, and 3. Each point on the graphs in Figure 1 represents the mean of the measurements of 100 spores; each point on the graphs in Figure 2 represents the mean of the measurements of the walls of 50 spores; and each point on the graphs in Figure 3 represents the quotient obtained by dividing the mean of the length measurements of 100 spores by the mean of the width measurements of the same spores. The points are connected by the solid line for *Cronartium ribicola* and the broken line for *Cronartium occidentale* in order to facilitate reading.

TABLE I.—*Field series; means for each 100 urediniospores measured; Cronartium ribicola*

Forest pathology No.	Host	Locality	Date collected	Mean length	Mean width	Mean wall thickness	Ratio mean length / mean width	Set No.
2607	Ribes vulgare	Font Hill, Ont.	June 6, 1915	22.7	14.6	1.61	1.56	1
23735	do	North Conway, N. H.	July 4, 1917					
34070	do	Red Bank, N. J.	Aug. 13, 1919					
23870	do	Rochester, N. Y.	Aug. 2, 1917					
22216	do	Chalmers, Mass.	Aug. 11, 1916					
34430	do	Pomroy, Minn.	July 26, 1919	22.6	14.7	1.60	1.54	2
23105	do	Little Compton, R. I.	Aug. 17, 1916					
23205	do	Acton Corner, Me.	Aug. 30, 1916					
23254	Ribes reclinatum	Bar Harbor, Me.	Oct. 2, 1916					
22443	do	Newport, R. I.	Sept. 5, 1916					
2592	do	Lyndonville, Vt.	Sept. 12, 1914	22.9	14.8	1.60	1.55	3
34260	do	Shafer, Minn.	Sept. 29, 1919					
23888	Ribes nigrum	Rochester, N. Y.	Aug. 18, 1917					
23012	do	Warwick, R. I.	July 24, 1916					
23846	do	North Whately, Mass.	July 26, 1917					
34442	do	Cannon City, Minn.	July 30, 1919	23.8	15.9	1.80	1.50	4
23902	do	Tupper Lake, N. Y.	Aug. 22, 1917					
24021	do	Red, Bank, N. J.	Aug. 21, 1918					
20623	do	Font Hill, Ont.	Aug. 13, 1915					
25337	do	Temple, N. H.	June 10, 1919					
23218	do	Limerick, Me.	Sept. 4, 1916	23.7	15.2	1.61	1.56	5
34036	do	Amery, Wis.	July 11, 1919					
23020	Ribes hirtellum	Lyman, Me.	July 25, 1916					
23734	do	Bath, N. H.	July 2, 1917					
34435	Ribes oxyacanthoides.	Pomroy, Minn.	July 28, 1919					
29632	do	Rice Lake, Wis.	Sept. 18, 1918	23.8	14.9	1.79	1.60	6
24043	Ribes cynosbati	West Greenwich, R. I.	Oct. 15, 1918					
24032	do	Bartlett, N. H.	June 13, 1918					
20695	do	Norfolk, Conn.	June 13, 1916					
2606	do	Font Hill, Ont.	June 26, 1915					
23868	do	Stephentown, N. Y.	Aug. 4, 1917	23.7	14.8	1.61	1.60	7
34446	do	Sec. 26, R. 21, Minn.	Aug. 1, 1919					
34103	do	Clear Lake, Wis.	July 23, 1919					
25334	do	Bethel, Vt.	July 6, 1919					
22137	Ribes americanum.	Central Village, Mass.	July 6, 1916					
24045	do	Font Hill, Ont.	June 22, 1917	23.6	14.9	1.70	1.58	8
23764	do	St. Johnsbury, Vt.	July 20, 1917					
34235	do	Munch Township, Minn.	Sept. 21, 1919					
34537	do	Downing, Wis.	Aug. 26, 1919					
22165	Ribes glandulosum.	Norfolk, Conn.	July 3, 1916					
23859	do	Bartlett, N. H.	July 28, 1917	23.9	14.6	1.82	1.64	9
28232	do	Brunswick, Me.	May 22, 1918					
23877	do	North Thetford, Vt.	Aug. 7, 1917					
24321	do	Amery, Wis.	Aug. 11, 1916					
24019	Ribes triste	Tuckerman Ravine, N. H.	Aug. 3, 1918					
23365	do	Rush Lake, Minn.	June 14, 1918	24.0	15.0	1.68	1.56	10
34095	Ribes odoratum.	Petersham, Mass.	July 30, 1919					
23125	do	Kittery, Me.	Aug. 18, 1916					
23834	do	Keeseville, N. Y.	July 24, 1917					
23798	do	Orford, N. H.	July 11, 1917					

TABLE II.—*Field series; means for each 100 urediniospores measured; Cronartium occidentale*

Forest pathology No.	Host	Locality	Date collected	Mean length	Mean width	Mean wall thickness	Ratio mean length / mean width	Set No.
27029	Ribes aureum	Farmington, N. Mex.	Sept. 9, 1918	24.2	15.4	2.17	1.57	1
27033	do	Aztec, N. Mex.	Sept. 10, 1918					
27151	do	Clayton, N. Mex.	Sept. 26, 1919					
27154	do	Farmington, N. Mex.	Aug. 2, 1919					
29087	do	Miller Valley, Ariz.	Oct. 6, 1917					
28218	do	Monrovia, Calif.	May —, 1919	26.6	15.7	2.17	1 69	2
28275	do	do	May 13, 1919					
29518	do	Morgan, Utah	Aug. 24, 1920					
29519	do	Iron Co., Utah	July 30, 1920					
26475	Ribes odoratum	Prescott, Ariz.	Oct. 2, 1917					
29515	Ribes aureum	Fillmore, Utah	Aug. 7, 1920	26.0	15.2	2.11	1.70	3
29516	do	Hygrum, Utah	Aug. 22, 1920					
29517	do	Scipio, Utah	Aug. 10, 1920					
36221	do	Iron Co., Utah	July 30, 1920					
36222	do	Beaver, Utah	Aug. 3, 1920					
27100	do	Hayden, Colo.	Oct. 18, 1918	25.3	15.9	2.17	1.58	4
26159	do	Meeker, Colo.	Oct. 2, 1917					
27046	do	Trimble Hot Springs, Colo.	Sept. 14, 1918					
27047	do	Hermosa, Colo.	Sept. 15, 1918					
27049	do	Durango, Colo.	Sept. 15, 1918					
32707	Grossularia velutina.	Mono Lake, Calif.	Aug. 20, 1919	23.9	15.2	1.91	1.57	5
34076	Ribes aureum	Bridgeport, Calif.	Aug. 2, 1919					
36621	Ribes gracillimum.	do	Aug. 20, 1919					
34019	Grossularia inermis.	Denver, Colo.	July 1, 1919					
34020	do	do	July 1, 1919					
29525	do	Monticello, Utah	Aug. 15, 1918	24.1	15.3	1.90	1.58	6
29654	Grossularia reclinata.	Mancos, Colo.	Sept. 12, 1918					
22644	do	Prescott, Ariz.	Oct. 27, 1917					
29392	do	Mancos, Colo.	July 31, 1918					
26745	Ribes aureum	Pagoro Springs, Colo.	Sept. —, 1917					
22648	Ribes odoratum	Prescott, Ariz.	Oct. 29, 1917	25.7	15.4	2.08	1.67	7
22635	do	do	Oct. 27, 1917					
29463	do	Mancos, Colo.	Aug. 9, 1918					
22634	Ribes aureum	Prescott, Ariz.	Oct. 27, 1917					
36112	do	Monrovia, Calif.	June 15, 1920					
28294	Ribes gracillimum.	Monrovia, Calif.	June 25, 1919	25.6	14.5	1.89	1.76	8
28277	Ribes aureum	do	May 18, 1919					
28276	do	do	May 17, 1919					
23396	do	do	May 22, 1919					
25054	Ribes odoratum	do	Oct. 27, 1919					
26485	Ribes aureum	Naturita, Colo.	Aug. 18, 1917	25.8	15.7	2.16	1.65	9
26499	do	Denver, Colo.	Sept. 25, 1917					
27101	do	Craig, Colo.	Oct. 18, 1918					
27039	do	La Plata, Colo.	Sept. 11, 1918					
36059	do	Monrovia, Calif.	Feb. 25, 1919					
2859	do	Boulder, Colo.	July —, 1914	25.6	15.3	2.06	1.68	10
24648	do	Ute Reservation, Colo.	July —, 1897					
22618	do	Bayfield, Colo.	Aug. 26, 1917					
24420	do	do	Sept. 15, 1917					
36089	do	Monrovia, Calif.	June 14, 1920					

TABLE III.—*Block Island series; means for each 100 urediniospores measured; Cronartium ribicola and Cronartium occidentale*

CRONARTIUM RIBICOLA

Forest pathology No.	Host	Locality	Date collected	Mean length	Mean width	Mean wall thickness	Ratio mean length / mean width	Set No.
34555	Ribes odoratum	Block Island, R. I.	June 22, 1920	24.0	15.0	1.80	1.61	11
34556	do	do	June 22, 1920	23.5	14.6	1.87	1.61	12
34579	do	do	July 20, 1920	23.6	15.5	1.57	1.53	13
34622	do	do	Sept. 8, 1920	22.4	15.1	1.83	1.49	14
34878	do	do	Sept. 30, 1920	24.0	15.0	1.74	1.60	15
34881	do	do	Sept. 30, 1920	23.8	15.8	1.73	1.51	16
34558	Ribes nigrum (Naples).	do	June 22, 1920	23.2	15.2	1.56	1.53	17
34619	do	do	Sept. 8, 1920	21.3	14.7	1.75	1.45	18
34888	Ribes americanum.	do	Sept. 30, 1920	21.6	14.6	1.73	1.48	19
34880	Ribes prostratum.	do	Sept. 30, 1920	23.4	15.6	1.73	1.51	20

CRONARTIUM OCCIDENTALE

34568	Ribes odoratum	Block Island, R. I.	June 23, 1920	25.7	14.6	2.39	1.77	11
34574	do	do	June 30, 1920	24.9	15.2	2.52	1.64	12
34630	do	do	Sept. 9, 1920	26.9	14.5	2.26	1.85	13
34634	do	do	Sept. 9, 1920	24.4	13.8	1.96	1.77	14
34846	do	do	Sept. 9, 1920	24.2	15.7	2.03	1.54	15
34877	do	do	Oct. 1, 1920	24.4	14.6	2.34	1.67	16
34627	Ribes nigrum (Naples).	do	Sept. 9, 1920	23.5	14.7	1.99	1.60	17
34871	do	do	Oct. 1, 1920	24.7	15.4	2.20	1.61	18
34647	Ribes americanum.	do	Sept. 9, 1920	25.0	15.5	2.07	1.60	19
34633	Ribes prostratum.	do	Sept. 9, 1920	24.5	15.0	2.08	1.63	20

TABLE IV.—*Washington, D. C., greenhouse series; means for each 100 urediniospores measured; Cronartium ribicola and Cronartium occidentale*

CRONARTIUM RIBICOLA

Forest pathology No.	Host	Locality	Date collected	Mean length	Mean width	Mean wall thickness	Ratio mean length / mean width	Set No.
34684	Ribes aureum	Washington, D. C.	July 15, 1920	22.0	15.0	1.84	1.47	21
34697	do	do	Aug. 2, 1920	25.5	15.2	2.24	1.67	22
34722	do	do	Aug. 25, 1920	24.3	16.1	1.99	1.51	23
34826	do	do	Oct. 8, 1920	25.6	17.7	2.29	1.44	24
36466	do	do	July 21, 1920	23.8	14.9	1.84	1.68	25
34712	do	do	Aug. 11, 1920	24.6	15.1	2.11	1.63	26
34740	do	do	Aug. 4, 1920	22.3	15.5	2.06	1.44	27
36125	do	do	July 8, 1920	23.3	14.5	1.95	1.61	28
36465	do	do	Aug. 9, 1920	25.4	16.1	1.87	1.57	29
36470	do	do	Aug. 9, 1920	23.7	14.3	2.01	1.66	30

CRONARTIUM OCCIDENTALE

36104	Ribes aureum	Washington, D. C.	July 1, 1920	24.0	16.1	2.19	1.49	21
36150	do	do	July 2, 1920	26.0	15.8	2.29	1.65	22
36233	do	do	Aug. 17, 1920	25.9	16.7	2.66	1.55	23
36406	do	do	May 22, 1920	25.0	15.5	2.22	1.62	24
36439	do	do	Aug. 2, 1920	24.0	15.4	1.99	1.57	25
36446	do	do	Aug. 9, 1920	25.3	15.3	2.05	1.65	26
36078	do	do	July 8, 1920	24.9	14.3	2.07	1.75	27
36131	do	do	July 14, 1920	25.2	16.7	2.11	1.51	28
36404	do	do	June 22, 1920	25.5	16.1	2.11	1.58	29
36429	do	do	June 9, 1920	24.2	15.3	1.93	1.58	30

TABLE V.—*Summary of measurement data; urediniospores of Cronartium ribicola and Cronartium occidentale*

Species	Length [a]			Width			Mean [b] wall thickness	Ratio mean length / mean width
	Mean length	Standard deviation	Coefficient of variability	Mean width	Standard deviation	Coefficient of variability		
Field series:								
Cronartium ribicola	23.5	2.6	11.1	15.0	1.8	12.1	1.70	1.57
Cronartium occidentale	25.2	3.5	13.7	15.3	1.8	11.4	2.05	1.64
Block Island series:								
Cronartium ribicola	23.1	2.6	11.3	15.1	1.6	10.8	1.73	1.53
Cronartium occidentale	24.9	2.9	11.8	14.9	1.7	11.5	2.18	1.67
Washington, D. C., greenhouse series:								
Cronartium ribicola	24.1	3.4	13.9	15.4	2.0	12.9	2.02	1.57
Cronartium occidentale	25.0	3.1	12.3	15.7	1.9	12.0	2.16	1.60

[a] The length and width means are based on 1,000 measurements for each species in each series.
[b] The mean wall thickness is based on 500 measurements for each species in each series.

DISCUSSION

Each of the three series of measurements serves a different purpose; for the mean measurements of the sets in the *Field series* evidently are better species indices than those in the two other sets, being based on a broader sampling system; and the means in the *Block Island series* show the difference in size of the urediniospores of the two species on a few Ribes hosts growing in the same locality, as well as the practical utility of 100 spore means for diagnostic purposes; whereas the means in the *Greenhouse series* represent the variation in the urediniospores of the two species on the same host (*Ribes aureum*) grown under experimental conditions in the greenhouse.

It is evident from the graphs of the length means and wall thicknesses that the two species are distinct, as far as these two factors are concerned, in the *Field series* and *Block Island series*, but that they are not separable on the same bases in the *Greenhouse series*. The respective value of the measurements for diagnostic purposes appears to be in the order of length mean, mean wall thickness, and ratio of mean length divided by mean width. The width mean has no diagnostic value. The results indicate that measurements of the urediniospores from hosts growing in the greenhouse should be used with extreme caution, if at all, in any attempt to diagnose field collections of either species. The data obtained in the *Field series* and the *Block Island series*, however, appear to warrant the conclusion that field collections can be separated in most cases on the basis of spore size, spore shape, and wall thickness. The following discussion of diagnostic division points is accordingly confined to the data from sets 1–20 inclusive.

The horizontal line drawn at 24.1μ, in Figure 1, separates 37 out of the 40 length means correctly, all 20 means for *C. ribicola* being below the line and 17 means for *C. occidentale* being above the line. One mean for *C. occidentale* falls on the line.

The horizontal line drawn at 1.88μ, in Figure 2, separates all 40 of the mean wall thicknesses correctly, the 20 for *C. ribicola* being below the line and the 20 for *C. occidentale* being above the line.

The horizontal line drawn at 1.59, in Figure 3, separates 29 out of 40 of the ratios of mean length divided by mean width correctly, 14 out of 20 for *C. ribicola* being below the line and 15 out of 20 for *C. occidentale* being above the line.

In other words the diagnostic division points,

24.1 for the length mean,
1.88 for the mean wall thickness, and
1.59 for the ratio of mean length divided by mean width,

would have correctly identified 92.5 per cent, 100 per cent, and 72.5 per cent, respectively, of the sets measured.

Examination of the graphs shows that the measurements for *C. occidentale* in set 5 are abnormal, that is, on the wrong side of the diagnostic division points for both length mean and ratio; that the figures are low for the length mean of the same species in set 6, low for the wall mean in sets 5 and 6, and below the line for the ratio in set 6. However, there is not a single case where the figures for any one set for either species fall on the wrong side of the line for all three criteria, and only one case, the one in set 5 men-

tioned above, where the figures fall on the wrong side for two criteria. On account of the greater irregularity in the ratio figures, a variation above or below the line is of less importance than a similar variation in length or wall thickness mean. If the two latter criteria only are considered there are but 2 means out of 40 which are definitely out of line—the length means for *C. occidentale* in sets 5 and 17—which makes the biometric diagnosis correct in 95 per cent of the trails.

The relation of the means of sets 1 to 20 to the diagnostic division points for the length mean and for the ratio of mean length divided by mean width is shown again in Figure 4, a type of figure which appears to be particularly satisfactory for illustrating the application of the criteria of size and shape to biometric diagnosis. Each of the plotted points represents both the mean length and mean width of 100 measurements. The means for *C. ribicola* are shown as dots and the means for *C. occidentale* are shown as circles. Set numbers have been omitted purposely, but the position of the means for any set can be found by reference to the mean values given in Tables I to III. For example, the means for set 1 are indicated by the dot and circle at 22.7 and 14.6, and at 24.2 and 15.4, respectively.

The diagnostic division point for the length mean, represented by the horizontal line drawn at 24.1 in Figure 1, is here represented as a vertical line at 24.1. The diagonal line, drawn to satisfy the equation $y=\frac{x}{1.59}$, represents the diagnostic division point for mean length divided by mean width. The figure illustrates clearly the "scatter" or shotgun pattern of the mean sizes for each of the 100's measured, the fact that length is the factor governing the distribution of the points to the left or right of the 24.1 line, and the effect of variation in size on the position of the plotted points with respect to the diagonal.

The difference between the 1,000 spore length means of the two species (see Table V) for the *Field series* is 1.76 ± 0.092, for the *Block Island series* 1.82 ± 0.072, and for the *Greenhouse series* 0.81 ± 0.099. No attempt will be made at present to establish the significance of these differences with respect to their probable errors, since it seems preferable to leave any such discussion as well as a more detailed description of the mounting measuring and analysis methods to a later paper.

The ranges for the spore sizes of the two species are given in Table VI. It is obviously impossible to separate the species on the basis of the range of the spore sizes. Ranges obtained by subtracting and adding the standard deviation to the mean for each dimension—which might be called "standard ranges"—are, however, more significant. These ranges in the three series are given in Table VII. In spite of the fact that the standard ranges are much more accurate indices of the spore sizes than the complete ranges, they do not serve to bring out the differences between the species as clearly as the tabular or graphic representation of the means.

It must be admitted after examination of the graphs in Figures 1 and 2 that *C. ribicola* and not *C. occidentale* is the species which appears to be "abnormal" under greenhouse conditions. No adequate explanation of the "abnormality" is possible at present.

In the *Field series* the measurements of 5 lots of 20 spores each were grouped together, as has been previously stated,

TABLE VI.—*Ranges for length and width of the urediniospores of Cronartium ribicola and Cronartium occidentale*

Series	C. ribicola		C. occidentale	
	Length	Width	Length	Width
Field	16–34	10–22	16–37	10–21
Block Island	13–32	10–21	18–37	10–22
Greenhouse	15–40	10–24	16–39	10–23

TABLE VII.—*Standard ranges for the length and width of urediniospores of Cronartium ribicola and Cronartium occidentale*

Series	C. ribicola		C. occidentale	
	Length	Width	Length	Width
Field	20. 9–26. 1	13. 2–16. 8	21. 7–28. 7	13. 5–17. 1
Block Island	20. 5–25. 7	13. 5–16. 7	22. 0–27. 8	13. 2–16. 6
Greenhouse	20. 7–27. 5	13. 4–17. 4	21. 6–28. 4	13. 5–17. 3

and the means for length and width illustrated in Figure 1 were based on the resultant 100's. This procedure undoubtedly resulted in a set of means showing less variation than would be expected if the means had been based on the measurements of 100 spores from single specimens, in which case there would have been more danger of crossing the dead line 24.1. The points on the graph for the length means of sets 1–10 in the *Field series* are, therefore, not exactly comparable with the points for sets 11–20 in the *Block Island series*. As a matter of fact, the *Block Island series* is the only one of the three which illustrates the way in which biometric diagnosis methods based on measurements of 100 spores might work out when applied to unknowns.

The data indicate that 200 measurements would have been better than 100 as a basis for determining the means; and, in cases where the species are close together as these rusts are, it would seem wiser to use the higher number. Means based on 200 measurements would probably have shown less tendency to cross the diagnostic division point than the means based on 100 measurements.

It would be preferable also in diagnosing unknown specimens to take spores from as many different leaves or sori as possible for each specimen studied; in other words, to sample the specimen with the aim of getting the best possible representative lot of spores for the mount. Unfortunately it was not practicable to follow this course in all the cases reported in this paper.

The following examples indicate the futility of attempts to use measurements of fresh spores as size standards, particularly in the case of *C. ribicola*, unless, of course, they are to be compared with other measurements made on fresh spores. The mean measurements for 137 fresh urediniospores of *C. ribicola* from *Ribes gracillimum*, mounted in water, were found to be 28.7 by 18.4, with a standard range of approximately 25 to 33 by 16 to 23μ. The walls of this set were not measured. The means of 50 fresh urediniospores, mounted in water, from a specimen collected on *Ribes aureum* growing in the greenhouse were 33.2 by 21.5μ, with a standard range of approximately 30 to 37 by 20 to 24μ. The mean wall thickness was 0.91μ. These means and standard ranges are far out of line with the figures given in the tables for *C. ribicola*. On the other hand the means for 330 urediniospores from herbarium specimens of *C. ribicola* collected in the field on various hosts were 22.3 by 15.4μ, with a standard range of 18 to 27 by 13 to 18μ. The mean wall thickness was approximately 2.00μ. These figures agree fairly well with those for sets 1, 2, and 3 of *C. ribicola* in Table I, except for the wall mean; yet the results are not really comparable, except in a very general way, because the measurements were made some years ago by methods which were not comparable with those used by the writer.

On the basis of the data presented, it would be going too far to expect biometric diagnosis methods to yield 100 per cent correct results; but they are the only methods applicable in cases where one is dealing with herbarium material, and where inoculation experiments are impossible. The diagnostic division points given in this paper can not be expected to hold good unless the mounting methods are rigidly followed. The measuring must be done with great care—as all real measuring should be done—and the measurements should be analyzed by statistical methods. Under such conditions the investigator can separate the uredinial stages of *C. ribicola* and *C. occidentale* in most cases with comparative ease.

In a later paper a biometric comparison of the aeciospores of the two species will be presented.

SUMMARY

The above analysis of measurements on 3,000 urediniospores of *Cronartium ribicola* and *Cronartium occidentale* indicates that the two species may be separated in the uredinial stage with practical certainty on the basis of spore size, shape, and wall thickness.

The most important criteria for biometric diagnosis are the length mean, the mean wall thickness, and the ratio of mean length divided by mean width.

The diagnostic division point for the length mean is 24.1μ, for the mean wall thickness 1.88μ, and for the ratio of mean length to mean width 1.59.

In the cases of collections from the field and from experimental plots these three diagnostic division points proved good in 92.5 per cent, 100 per cent, and 72.5 per cent, respectively, of the trials.

The dimensions of urediniospores produced under greenhouse experimental conditions do not appear useful for distinguishing these two species.

The data presented in the paper are applicable only when the conditions of mounting, measuring, and analysis are strictly comparable.

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ROOT ROT OF PEAS IN THE UNITED STATES CAUSED BY APHANOMYCES EUTEICHES (N. SP.) [1,2]

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INTRODUCTION

During the past five years, in which the senior writer has endeavored to determine the causes which produce the root rot of peas known widely in the United States, several fungous diseases have been distinguished which severally or together appear to be responsible for all of the important injury which has been found incident to the underground portion of this plant. One of the less important of these diseases caused by a species of Fusarium has been described in a previous paper (*8*).[3] The most important of these diseases, more important, in fact, than the others combined, is the subject of the present paper. The account of the morphology and taxonomy of the fungus causing the disease and the drawings are contributed by the junior author. Several fungous diseases of pea roots of much local importance have also been studied. Among these are two caused by species of Pythium, one of which in some seasons is almost always associated with Aphanomyces, an association which misled the senior writer in a previous note (*6*) to ascribe to the Pythium alone the injury due to both organisms. The diseases caused by species of Pythium remain to be described in a following paper.

THE DISEASE

DESCRIPTION

The injury caused by a fungous parasite which invades only the subterranean portion of the plant must, of course, be sought in its early stages in the roots themselves. In later stages the top of the plant may be modified in response to the root injury in a way which may be specifically characteristic. In the case of this disease the injury eventually presented by the top of the plant is not characteristic, the form which it takes depending largely upon the stage of development at which the roots became thoroughly invaded and to a lesser extent upon the degree of resistance of the variety of peas. If the plant becomes invaded in the basal stem below ground as well as in the roots before the plant has developed more than three or four nodes, sudden wilting may result. Usually under field conditions invasion takes place later than this, and the result is a general retardation of growth, the death of the lower leaves progressively upward, and finally, when the plant is in full bloom, it may shrivel up completely. More frequently, however, the plant persists in a weakened condition until it has brought its poorly filled pods to maturity.

If extensive infestation of the roots is delayed until the blossoming period, the plants may mature under favorable conditions without any conspicuous indication of injury. These several symptoms have no common factor which distinguishes plants infected with Aphanomyces from those attacked by several other fungi. However, there is one test which can be applied that will often give a decisive indication of this disease. If some of the infested plants are pulled, the stems of those which are thoroughly invaded will fail to break at the seed, as is the usual rule with healthy plants, but the vascular core of the taproot will pull out as a long string

[1] Received for publication May 19, 1924—issued April, 1925.
[2] Results of work done in cooperation with the Agricultural Experiment Station of the University of Wisconsin.
[3] Reference is made by number (italic) to "Literature cited," p. 325.

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with a few of the larger lateral branches. No other type of root rot has been found which permits the pulling out of these vascular strings. This test does not apply in the earlier stages of disease and sometimes fails to give results when the soil is exceedingly hard and dry. Examination of the roots themselves will reveal the extent of injury by the soft decay of the cortex. In early stages there is a pale yellow discoloration of the decayed tissue not readily distinguishable from the discoloration associated with the invasion of the mycorrhizal fungus described in a previous paper (*9*).

The soft decay and shrinking of the cortex of the roots and of the portion of the stem below ground usually distinguishes this disease from the turgid roots with the mycorrhizal fungus. After a time the dead cortical tissue usually becomes blackened (pl. 1) so that it can not readily be distinguished by this character from other forms of root decay. If the visible symptoms already described fail to distinguish the disease with certainty, it is almost always possible at any stage in the development of the disease to distinguish it by an examination of the roots with a microscope. The characteristic spores of the fungus, described later, are always formed more or less abundantly in some of the dead cortex of root (pl. 2) or more rarely in that of the stem, and serve to determine the presence of this parasite beyond possible doubt.

HISTORY OF THE DISEASE

Since this disease has not been distinguished previously under any name, it has no unmistakable written history. There are a few references to pea-root troubles, ascribed to other fungi, but on such insufficient evidence that it is not impossible that they may be pertinent here. The first of these is by Wittmack [4] (*19*), who found in decaying roots of peas sent to him by Sadebeck from Hamburg, Germany, oospores apparently belonging to a new Pythium, which he called *P. sadebeckianum*.

In the United States the disease has undoubtedly been present for a long time and has compelled the abandonment of intensive culture of peas for canning purposes in certain restricted areas. Recently Clinton (*2*) has described a root rot of peas in Connecticut evidenced by the presence of oospores which he believed to be those of *Phytophthora cactorum*. The senior writer has been unable to produce any extensive decay in pea roots growing in soil with a culture of this species, and in no case were oospores formed in the few small lesions produced. Although infection of root ends with a pure culture of a species of Pythium has been obtained by the writer with the formation of oospores in the decaying tissue, and though spores apparently of this species have been found occasionally in field material, nevertheless the measurements and drawings given by Clinton appear for the most part to agree more closely with the spores of the Aphanomyces species, to which reference was made in a brief abstract (*7*) and which is fully described in this paper, than with those of any other fungus that has been encountered. There are other references to rootrot of peas in the United States which undoubtedly indicate this disease, but which do not contain a sufficiently adequate description to make exact determination possible.

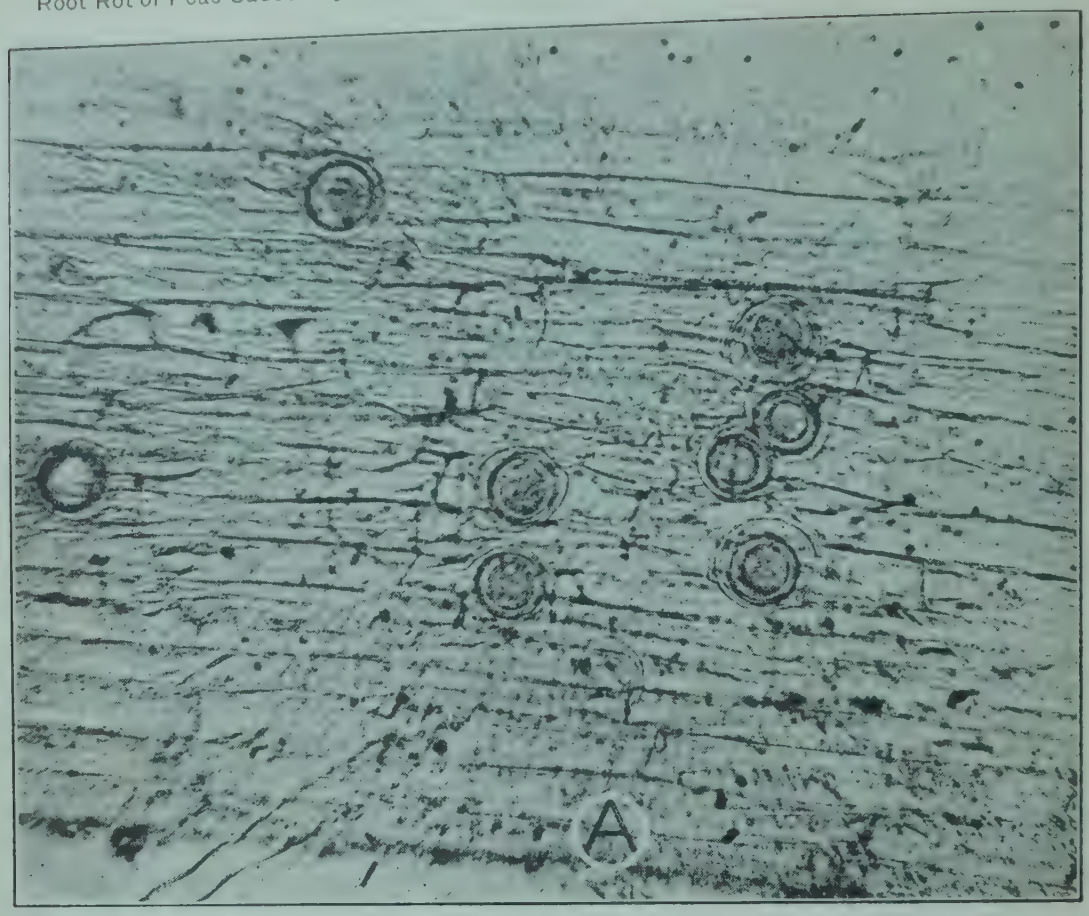
THE FUNGUS

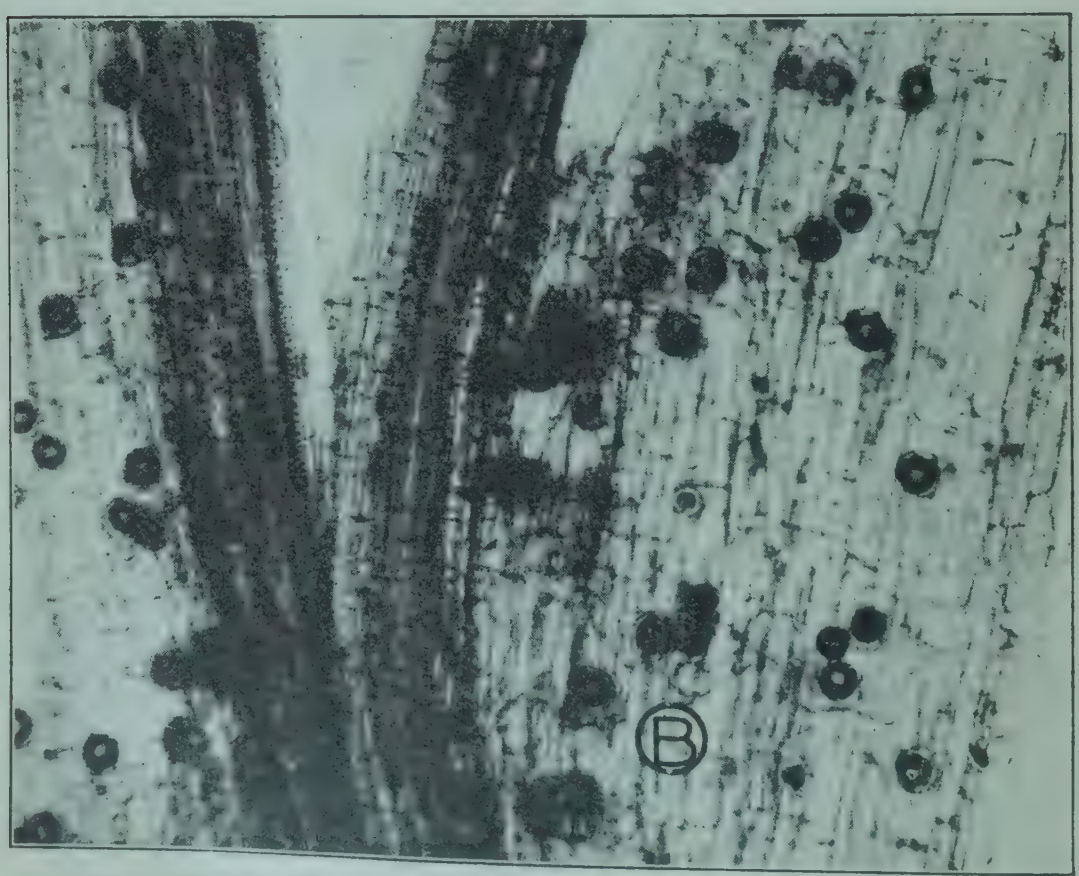
HOST PLANTS

Although it seems unlikely that this fungus is parasitic only on peas, a search for other host plants has thus far been in vain. The roots of many species of plants growing among diseased peas have been examined for the presence of the characteristic oospores and decayed cortex which might have been produced by this fungus. In order to test thoroughly the possible relation of this fungus to the species of Aphanomyces on sugar beets, described by Peters (*13*, *14*), seedlings of this plant and of cress (*Lepidium sativum*) have been repeatedly inoculated without any infection resulting. The conclusion that these fungi are distinct, at least in their physiological capacities, is further borne out by the fact that examination made of fields of beets grown on pea-sick soil, made both by the senior writer and R. E. Vaughan, has discovered no trace of the European disease. On the other hand, although it appears generally true that beets grow well on old pea fields and vice versa, experience is reported otherwise in the Salt Lake Valley, Utah. It may be of interest to note in this connection that in the course of making isolation from diseased peas from this valley with the

[4] WITTMACK, L. UEBER EINE DURCH PYTHIUM VERANLASSTE KRANKHEIT DER ERBSENWURZELN. Paper presented at 64. Versamml. zu Halle, Gesell. Deut. Naturf. u. Aerzte, Sep. 1891. Not published. Title in Verhandlungen 2: 108. 1892.]

Root rot of peas, variety Early Market, caused by *Aphanomyces euteiches* occurring in a field at Hanover, Md., May 21, 1920. The plant at the left with white clean stem and turgid roots is not infested. The three remaining plants show increasing degrees of disease progressively toward the right. The plant at the extreme right shows the first symptoms of disease above ground in the shriveled lower leaf





Oospores of *Aphanomyces euteiches* in cortex of the roots of peas inoculated with a pure culture of the fungus

A.—Oospores in a razor section. × about 300
B.—Oospores as they appear in a decaying rootlet crushed under a cover glass. × about 150

writer, John W. Carlson, of the Utah Experiment Station, obtained among other fungi a culture of *Pythium (Rheosporangium) aphanadermatum* (Edson) Fitzpatrick, one of the well-known sugar-beet parasites. This is one of the species of Pythium which the writer has found capable of producing root rot of peas, and if it is widely distributed in this valley it may be the common parasite of these plants which renders pea growing on old beet fields unprofitable.

GEOGRAPHIC DISTRIBUTION AND ECONOMIC IMPORTANCE

In the United States the disease has been found in practically every pea-growing district that has been searched with care. In the Eastern and Central States it occurs frequently, and often very destructively. It has been found in Utah, Idaho, and Montana, where it appears to be unimportant at present except under special conditions described later. In the Pacific Coast States it has been found but once, in diseased pea plants sent from Santa Clara, Calif. It appears that, although the disease is very widely distributed, it requires special soil and climatic conditions generally present only in the Eastern and Central States in order to become important when peas are grown intensively. It is probably this disease more than any other factor which has compelled the growing of peas in a comparatively long rotation, and has thus limited the culture of this food crop. Certain it is that were it not for the accumulation of this disease and others with intensive pea culture, the cost of producing canned green peas and probably of dried peas would be greatly reduced. In the absence of any accurate survey of fields in the region where this disease occurs, it is impossible to state approximately the number of acres that are damaged or destroyed each year; in some of the older districts in unfavorable years as much as 25 per cent of the acreage seems to be infested. From the reports of growers and county agents, combined with a limited personal survey, it appears that several thousand acres of peas are rendered unprofitable or destroyed in the United States each year.

ISOLATION OF THE FUNGUS

The fact that this important parasite of peas has remained undescribed so long is undoubtedly due to the difficulties encountered in isolating it in pure culture. One cause of failure is due to the brief period of time during which the fungus flourishes in an active vegetative stage at any point in the host tissue. When the fungus first begins to invade tissue, it can hardly be induced to grow out on the culture medium in preference to the living cells. Only a few days are required at ordinary temperatures to exhaust and destroy the host cells, whereupon the mycelium begins to transfer its contents into the large oospores, which are formed abundantly. After this process is well under way it is again almost impossible to secure growth on culture media. Thus it is necessary to obtain diseased plants in which the fungus is growing vigorously just prior to extensive oospore formation if success in isolation is to be attained.

Another cause of failure is due to the large number of vigorous saprophytes which follow closely the invading Aphanomyces. Besides abundant bacteria, in some seasons there is almost always present a species of Pythium so much more vigorous in growth upon culture media that it usually submerges the Aphanomyces. As yet no method of surface disinfection tried has given material aid in destroying the saprophytes present. The parasite is extremely sensitive to bichloride of mercury, and its use almost invariably brings failure.

In order to secure cultures of the fungus from localities remote from facilities for making isolations or from plants which are so far decayed that direct isolation is impossible, the senior writer has been accustomed to pack diseased roots with soil from the field in tin cans kept tightly sealed awaiting a convenient time in which to combine this material with steam-sterilized soil. In this soil mixture peas are then grown properly protected from outside infection until plants are obtained which have developed the stage of the disease suitable for the isolation of the parasite. In this way cultures have been obtained for comparison from nearly all of the regions where the disease has been found. Fragments of tissue are selected in which the mycelium of the fungus is seen under the microscope to be filled with granular contents, are thoroughly washed in sterile water, and are placed on plates of 2 or 3 per cent clear agar, or preferably prune agar, as recommended by Hartley (4) for the isolation of Pythium. The parasite always grows sparsely, sending out long, straight filaments with comparatively short lateral branches through or over the agar. It will almost always outgrow the bacteria which soon develop abund-

antly. Fragments of agar containing ends of these strands are cut from the plate and transferred to new plates until no bacterial growth accompanies the fungus, when it is transferred to corn-meal agar for a stock culture. It often happens that strands of Pythium grow in a manner resembling Aphanomyces so closely that they can not be distinguished until they are transferred to new plates of prune agar, where they soon develop a white matted growth very different in character from the sparse arachnoid growth of Aphanomyces. Oftentimes many fragments of roots and stems must be selected and plated before a culture of the desired fungus is obtained.

MORPHOLOGY OF PARASITE

MYCELIUM

In longitudinal sections of diseased pea stems the vegetative stage of the parasite is revealed as a hyaline nonseptate mycelium, composed of hyphae, varying considerably in diameter among themselves, but individually not subject to abrupt fluctuations in respect to this dimension. (Pl. 3, A.) Branching is exhibited in moderate, usually not in great abundance, the branches generally being produced at angles approaching a right angle. Not infrequently branches show little linear growth, then remaining as short diverticulate spurs on the axial filaments. The fungus is largely intracellular, the hyphae being oriented longitudinally within the cells, their development between the cells being relatively meager and apparently more or less accidental. Appearances often suggest that the fungus passes through the cell walls of the host perhaps with less ease than, for example, some parasitic species of Pythium. Whereas in cortical tissue invaded by the common damping-off fungus, the hyphae pass promiscuously from cell to cell, without much evidence of the membranes providing any obstacle, in tissue invaded by the root rot parasite cells crowded with mycelium may lie adjacent to others entirely free of the fungus. It should be mentioned, however, that the cortical tissue invaded by the root rot parasite is of a distinctly less succulent character than the cortical tissue of seedlings of various hosts subject to damping-off, and that strains of Pythium effective in producing the latter type of injury exhibit little or no aggressiveness toward pea plants readily attacked by the species of Aphanomyces under consideration. A somewhat similar mycelial distribution was reported by Weatherwax (*17*) in filaments of *Spirogyra dubia* Kg. invaded by *Aphanomyces phycophilus* DeBary.

SEXUAL REPRODUCTION

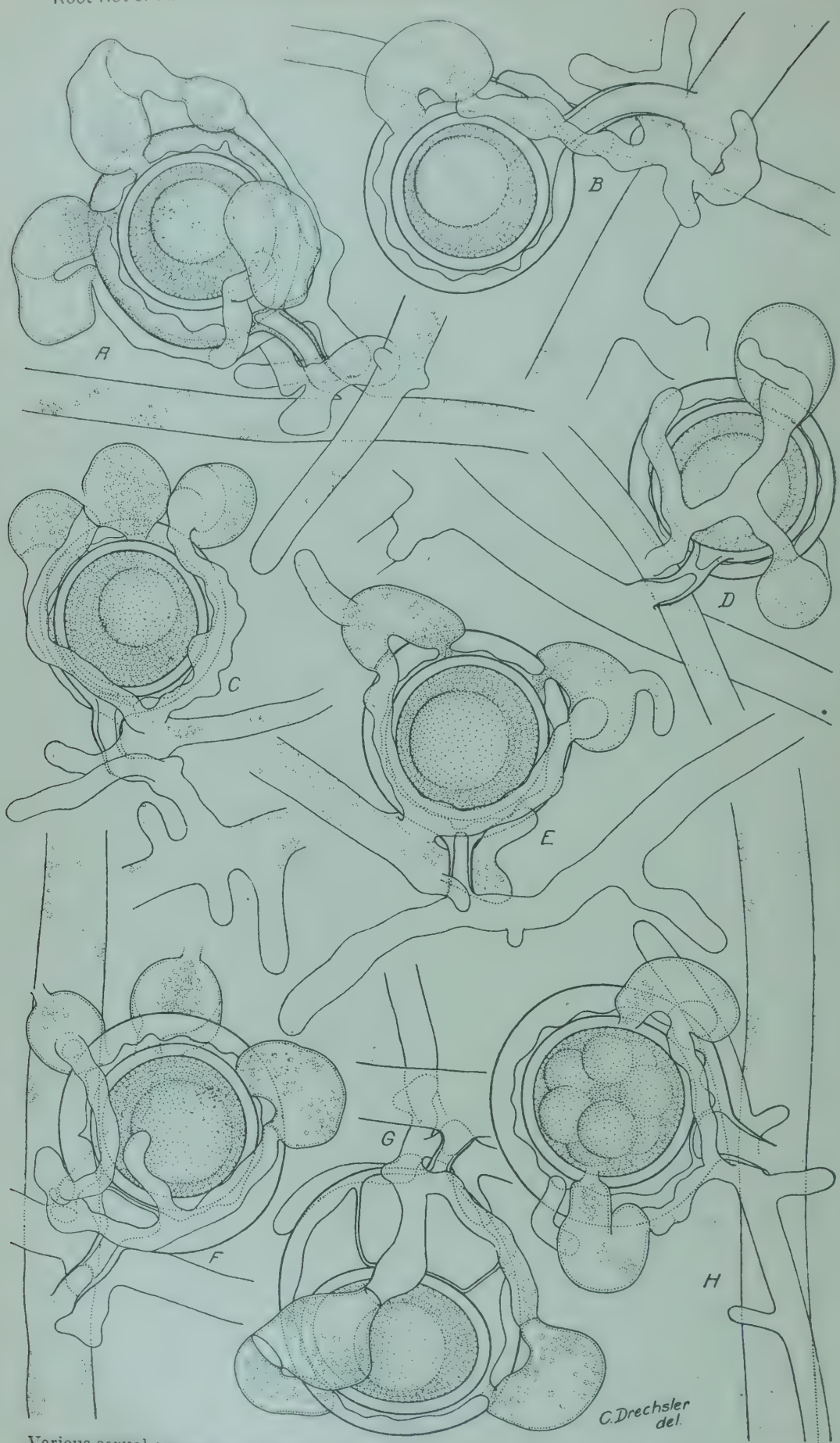
The purely vegetative condition represents a rather brief phase in the development of the fungus, mycelial growth coming to a pause as the cortical tissue begins to collapse. Oogonia and antheridia now make their appearance. Owing to the crowding of the hyphae within the host cells, the relation between the branches bearing these organs can not usually be distinguished. There is no reason to believe, however, that the sexual apparatus in diseased plants shows any significant departure from these structures as they develop on suitable artificial substrata where they can be accurately studied. The oogonia before fertilization are thin-walled, subglobose bodies with densely granular, vacuolate contents. After fertilization the oogonial wall becomes conspicuously thickened, the thickening being subject to peculiar irregularities, with the result that the inner contour is represented by a more or less sinuous line, giving the whole structure a peculiar internally scalloped appearance. (Pl. 4, A to H.) As in other species of Aphanomyces, the oogonial cavity is very largely but not completely occupied by the single oospore, a subspherical structure with a thick colorless wall, the thickness of the latter not given to great variations either with respect to different individuals or with respect to different portions of the same individual. The contents of the normal mature oospore consists of a

EXPLANATORY LEGEND FOR PLATE 3

A.—Portion of longitudinal section of basal part of pea stem in an early stage of infection, showing the development of the fungus in certain cortical cells and extension into adjacent cells. The ellipsoidal body near top of figure represents a developing oogonium. From material of a plant grown in soil inoculated with a pure culture of the parasite, killed in Flemming's weaker solution, embedded in paraffin, cut on the microtome, and stained with Flemming's triple combination. ×470

B.—Epidermis of hypocotyl of pea seedling derived from a surface-sterilized seed planted on sterile water agar in a test tube, the resulting culture inoculated 5 days after planting with a pure culture of *Aphanomyces euteiches*. Showing penetration by 3 extramatrical hyphae into tissue of plant—one, *Ba*, entering close to or at the juncture of adjacent epidermal cells; another, *Bb*, entering between two epidermal cells; and a third, *Bc*, entering between a guard cell and an epidermal cell. ×470

(For explanatory legend see p. 298)



Various sexual apparatus of Aphanomyces euteiches from 20 day old hard corn-meal agar cultures, illustrating close approximation of antheridial and oogonial stalks; branching of antheridial stalk; size, shape, and interrelation of oogonia and antheridia; character of cross walls setting of sex organs; and occurrence of diverticulate branches. Drawn with the aid of a camera lucida at a magnification of 1,580 diameters, and reduced in reproduction to a uniform magnification of 470 diameters

large, spherical, somewhat eccentric, vacuole-like body of apparently homogeneous structure, surrounded by a matrix containing numerous small uniform granules in regular concentric arrangement. The literature on the genus Aphanomyces like that on the Saprolegniaceae in general, shows some difference of opinion with reference to the nature of these internal elements, the large central body sometimes being referred to as an oil globule (*1*, *3*, p. 160); while evidently at other times (*3*, p. 10) it is regarded as being of a protoplasmic nature and the granule-like structures as consisting of oily matter. Since the small peripheral granules stain well with Sudan III in the species under consideration, it would seem the latter view is probably more nearly correct, although the apparent homogeneity of the material constituting the central globule can not be regarded as characteristic generally of protoplasm.

The relationships of the sexual apparatus can be studied to excellent advantage when the fungus is grown on hard corn-meal agar. By cutting off a thin surface layer of the substratum, a thin preparation is obtained containing the thallus at nearly a uniform optical level without any serious disruption of the structures concerned. Typically, and at least in the large majority of cases, the male and female organs arise from hyphae which show no close organic connection. The oogonium is apparently always terminal, being generally borne on a rather short stalk arising as a lateral branch from a hypha usually of more than ordinary diameter. The antheridial branch generally arises from a less stout filament, which in many instances will be found to cross the larger filament close to the points from which the sexual branches have their origin. (Pl. 4, A to H.) Usually the antheridial stalk becomes intimately involved with the oogonial stalk after making a partial turn about the latter, although the condition described by Von Minden (*11*) for his *Aphanomyces helicoides*, and figured by Kasanowsky (*10*) in his account of *A. laevis*, in which the antheridial stalk makes several distinct turns about the oogonial stalk, has never been found realized. A number of short diverticulate branches often are borne on the hypha from which the antheridial branch originates (pl. 4, A, B, E, H) and occasionally on the hypha to which the oogonial stalk is attached. (Pl. 4, C, E.) As far as can be determined, they serve no special purpose, but add to the characteristic involved appearance of the apparatus, thereby further enhancing the optical difficulty in resolving it with certainty.

The antheridial branch may be simple or branched, the branching frequently occurring near the base of the oogonium or a short distance from the base. Usually it is limited to a single bifurcation (pl. 4, B, H), but instances in which one (pl. 4, A) or both (pl. 4, F) of the resulting elements branched again have been found, one of the ultimate elements then sometimes failing to develop any male organ. (Pl. 4, D, F, G.) The antheridium is always an expanded structure set off from the stalk by a septum, which is frequently curved with the convexity protruding into the interior. Often the antheridium appears sharply arched in the manner of a measuring worm, the fertilization tube in such instances usually being produced in the region where the distal lobe is in contact with the oogonium. (Pl. 4, B, H.) Not rarely a hyphal diverticulum is present as a dorsal appendage (pl. 4, E, F) quite similar in appearance to analogous protuberances that can be observed occurring singly on the male organs of some species of Pythium and, as in the latter forms, apparently not serving any evident purpose.

In some instances when the antheridium attains unusually large proportions a transverse septum may be inserted, generally at a constriction. (Pl. 4, A, G.) The resulting structure may evidently be regarded either as two male organs developed in series on the same stalk or as a compound antheridium. Sometimes both of the male elements thus delimited have been observed communicating with the interior of the mature oogonium by independent tubes or apertures in the oogonial wall. While the origin of the antheridial branch near the base of the oogonial branch and a sort of contact relation of the two are of common occurrence and characteristic of the fungus, male stalks altogether unrelated to the oogonial stalk in place of origin also occur. As the number of antheridia to an individual oogonium varies from one to four, or even five, considerable variety in origin may be expressed in a single sexual apparatus.

The oogonia and oospores developed in culture are quite similar to those found in diseased host tissue, but a few details not easily observed in the latter substratum may here be studied to advantage. Thus the peculiar thickening of the oogonial wall will be seen produced into the distal portion of the supporting stalk, diminishing rather markedly, so that the latter is repre-

sented in its proximal portion in an entirely unmodified form. An interesting variability is exhibited by the partition delimiting the oogonial cavity at the base. Usually it occurs as a rather inconspicuous cross wall inserted in the distal portion of the stalk, providing no marked interruption in the roughly subspherical interior surface of the oogonial envelope. (Pl. 4, A, F, H.) It sometimes projects into the interior as a convex columella-like structure, and in some cases such development may be so pronounced that the available space is greatly reduced, constraining the oospore to adopt a distinctly ellipsoidal shape, with the major axis transverse to the axis of the oogonium. (Pl. 4, G.) Such modification may perhaps best be regarded as a fortuitous morphological peculiarity rather than as an abnormality, being present in material derived from the diseased host as well as in culture and evidently not adversely affecting the vitality of the oospore.

The processes of fertilization are not as amenable to direct observation as might be desired, for although an abundance of antheridia and oogonia develop readily in liquid culture, and consequently can be obtained in Van Tieghem preparations, their contents appear to degenerate at an early stage, or an oospore will be produced of a patently abnormal character, exhibiting a promiscuous granular or irregularly vacuolate internal structure. Examination of preparations of corn-meal-agar cultures abounding in normal sexual conditions indicate that when several antheridia are present, as is usually the case, all or several of them may develop fertilization tubes. For example, out of three or four antheridia attached to an individual female cell, two or three may usually be found to communicate with the interior of the oogonium by openings through the thick oogonial wall. While not all of the antheridia provided with such communications appear devoid of contents, it is certainly not unusual to find two male organs from which the protoplasm has disappeared completely or almost completely. (Pl. 4, A, F, H.) It is readily apparent that such a condition might be brought about by degeneration quite as well as by evacuation of contents into the oogonium. In a cytological study of a congeneric species, Kasanowsky (*10*) found that after fertilization was effected by one antheridium the nucleus of a second antheridium was intercepted in its passage through the fertilization tube. No statement is made by this author whether or not any transfer of cytoplasmic material may take place from a second antheridium previous to the entrance of the nucleus into the tube and its interception. The empty condition of plural antheridia, frequently encountered, points to such possibility. It is even not inconceivable that where nuclear degeneration is as easily effected as in the sexual apparatus of the coenocytic type, to which Aphanomyces seems to belong, the entrance of a supernumerary nucleus may not bring about as impossible a cytological situation as sometimes has been assumed.

GERMINATION OF OOSPORES

The germination of oospores of Aphanomyces appears to have been recorded for only two species, DeBary (*1*) having observed the process in material of *Aphanomyces stellatus* De Bary that had been kept in water for three months, and Kasanowsky (*10*) in material of a form he designated as *A. laevis*, DeBary, after this had passed through a resting period of over seven months. In both cases a germ tube was produced which perforated the oogonial wall and developed into a mycelium. A difference in the accounts of these authors may be noted in the promptness with which branching of the germ tube occurred, that of *A. stellatus* giving rise to a large number of hyphae very soon after emerging, if not immediately, while in Kasanowsky's fungus ramification was delayed until the tube had attained a length of 300 μ.

Unlike oospores of the two species mentioned, those of the parasite attacking peas require no extended resting period. When material from 15-day-old corn-meal-agar cultures was transferred to hanging drops in Van Tieghem cells, a considerable proportion of the oospores germinated, the method of germination, whether by mycelial development as observed by DeBary and by Kasanowsky, or by the production of zoospores which has not hitherto been recorded for any member of the genus Aphanomyces, seemingly being dependent to a great extent on the amount of nutrient material incorporated in the preparation. Thus Van Tieghem cultures prepared from material without any preliminary washing or removal of particles of substratum quite invariably exhibited direct germination into a mycelium. When the material was first allowed to soak, and the bits of solid food material removed as completely as possible, indirect germination by means of zoospores predominated. The initial changes which take place during the first 24 hours appear to be the same, regardless of eventual developments. The

thick oospore wall disappears as such, being reduced to a delicate membrane, which yields to the enlargement of the protoplast and is pressed against the inner surface of the oogonial wall, with the result that the appearance is presented of the oogonial cavity being entirely filled with protoplasmic contents. These contents, moreover, no longer exhibit the geometrical arrangement of the resting condition, but show instead a more or less uniformly granular condition.

If the conditions are such as to favor direct germination, one or several proturberances are now thrust through the oogonial wall. (Pl. 5, G, H.) The communications established by the fertilization tubes seem to serve an important rôle as channels of egress, as the granular germ processes can frequently be clearly seen passing through them into the antheridial cavity and thence emerging by a perforation through the antheridial wall; and owing to the collapse of the empty antheridia and difficulties in observation, instances of such utilization of these aperatures are very probably even more numerous than could be definitely established. In many cases the germ tube immediately gives rise to a number of branches, usually aggregating about a dozen, which thus occur in rather crowded, bristling arrangement not at all typical of mycelial ramification in the species (Pl. 5, L, K); in other cases branching is delayed until the germ tube has attained considerable length, and the sort of ramification then exhibited is altogether comparable to that shown by the thallus of the fungus generally. (Pl. 5, I, J.) Manifestly the conditions here represented correspond to those found in the two organisms investigated by DeBary and Kasanowsky, respectively. In appearance the resultant structures bear some similarity to those produced by the two types of direct germination of, for example, the sporangia of certain species of Phytophthora. They may, indeed, plausibly be explained in the same way—vegetative development of the oospore as a single energid, on the one hand, as contrasted with the development of multiple energids resulting from division processes incident to abortive zoospore production, on the other.

When an oospore germinates by the production of zoospores, a single filament is produced which ceases elongation after attaining a length varying from 8 to 12 times the diameter of the oogonium. (Pl. 5, E.) The germ hypha regularly decreases in width toward the tip, the distal portion usually measuring about 4 μ in this dimension, or somewhat less than one-half the diameter of the basal portion. At this stage, when only approximately half of the oogonial contents have passed into the germ hypha, zoospore formation is initiated. In the germ hypha the process shows no departure from the usual course that has been described so frequently in the filamentous vegetative sporangia of other members of the genus, yielding from 6 to 10 cylindrical portions of protoplasm connected, at least for a time, by a greatly attenuated strand. (Pl. 5, A.) It appears probable that development is in the main acropetal, the divisions delimiting the two or three most distal portions having been observed to be initiated after the separation of the basal portions had been effected, the last division of all setting off the terminal portion. Within the oogonial wall the residual material which has become concentrated in a subspherical mass near the orifice of the germ hypha undergoes similar cleavage, as evidenced by its segregation into lumps that become increasingly distinct and after some mildly writhing movements assume individuality as independent subspherical protoplasmic masses. (Pl. 5, A.) Suddenly the tip of the germ hypha gives way and the protoplasmic masses escape one by one in rapid succession, each rounding up and encysting near the orifice. An interesting feature in the evacuation of the germ sporangium is that the globose bodies within the oogonium enter the germ hypha at the base to replace distal ones by streaming through the small aperture in the oogonial wall and assuming the cylindrical form of the filament. In the course of about 10 seconds the entire apparatus is emptied. (Pl. 5, B.) The discharged spores are scattered loosely about near the mouth of the germ hypha or collected in whole or in part in a loose irregular aggregation. (Pl. 5, C, D, and F.) They number generally from 13 to 18 (most frequently 15), depending apparently somewhat on the size of the oospore and the proportion of oversized individuals capable of producing one compound or two normal motile forms.

While sporangial germination of the oospore does not appear to have been recorded hitherto for any species of Aphanomyces, it may be mentioned that a somewhat analogous development was noted by Sorokine (*16*) in the germination of globose bodies belonging to *A. stellatus*, which he designated as conidia. An extensive rupture in

the thick wall of these bodies through which the germ tube made its exit and their considerably inferior length provide differences in detail. Direct germination by a germ tube was also described for these conidia, the resulting hyphae being of the more remotely ramifying type, generally characteristic of the growing mycelium.

ASEXUAL REPRODUCTION

It is presumably altogether safe to take for granted that the sexual spores developed in the tissues of the diseased host constitute the regular resting bodies of the fungus, by the germination of which the parasite is reestablished in successive seasons. That by the production of germ sporangia they are also the chief means by which the fungus extends its distribution in the soil, appears at least very probable, although, as is well known, dissemination of the aquatic members of the genus is effected by zoospores produced in sporangia of mycelial origin. For when infected pea tissue containing an abundance of the mycelium of the parasite is placed in water no extramatrical development takes place, the organism thus differing considerably in behavior from the amphibious species of Pythium or Phytophthora, for example, which are frequently found in similar relationship, as well as from the congeneric form reported by Peters (*13*, *14*) as causing root rot of sugar beets in Germany. The pea parasite, however, continues in its development of sexual spores apparently uninterrupted. But even if zoospores could be produced by such means it is not certain, in view of the brief time elapsing between full mycelial development and the initiation of sexual stages, that extensive zoospore formation from mycelial elements could frequently be expected in nature.

On the other hand, in artificial culture, the production of zoospores from the ordinary filamentous sporangia characteristic of the genus can be induced, and that in exceedingly great profusion. Following the well-known methods for cultivating aquatic forms, the parasite was grown in pea decoction made by adding from 8 to 10 freshly shelled peas to 100 cc. distilled water in an Erlenmeyer flask and sterilizing by autoclaving or intermittent steaming. Altogether satisfactory results were also obtained by the use of canned peas with some of the liquor in which they were obtained, as well as by employing about twice the number of dried peas to the same quantity of water. Within three or four days at ordinary temperatures an extensive submerged mycelium was produced, appearing in the liquid medium as a translucent nebulous mat. The whole growth was now transferred to a deep Petri dish, the peas removed, and the mycelium washed several times at intervals of about 15 minutes with changes of sterile water. For convenience in examination it was found desirable the last time to add only enough water to keep the mat submerged.

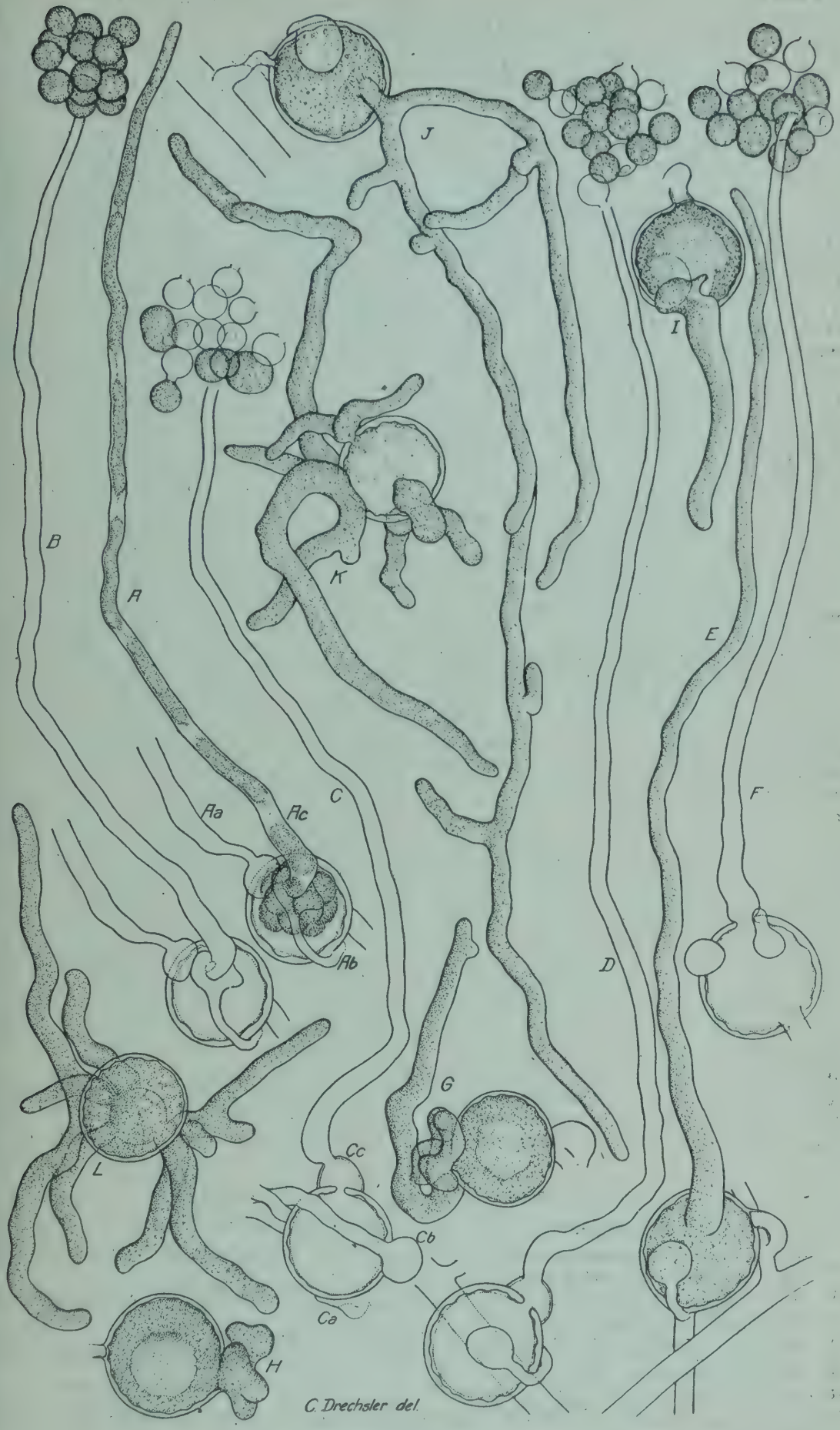
With young thalli at a temperature of about 20° C., evacuation of the sporangial filaments was found to begin about six to seven hours after washing was completed. As in the case of the germ sporangia, the internal developments follow the course described for congeneric species by other writers. It may not be superfluous however, to discuss certain matters which perhaps have not received adequate treatment hitherto, or which involve points in regard to which the fungus under consideration would appear to be at variance with its aquatic congeners.

For example, in the writings of most authors the distinction is made between vegetative hyphae and sporangia. While these structures are invariably said to be similar to each other in external morphology, the impression is conveyed that specialization in perhaps less obvious characteristics nevertheless obtains. Such a supposition, while conserving the analogy to other Saprolegniaceae, finds little support in the behavior of young vigorous thalli

EXPLANATORY LEGEND FOR PLATE 5

Oospores of *Aphanomyces euteiches* from 15 day old hard cornmeal agar cultures germinating in Van Tieghem preparations. × 470

A.—Germ sporangium 30 seconds before discharge
B.—Same sporangium 5 minutes later, after evacuation
C, D.—Evacuated germ sporangia showing utilization by germ hypha of aperture in oogonial wall produced by antheridium
E.—Oospore with single germ tube previous to separation of contents
F.—Evacuated germ sporangium
G to J.—Direct germination of oospores by production of hypha with ordinary type of branching
K, L.—Direct germination of oospore with close branching immediately after emergence of germ tubes



(For explanatory legend see p. 304)

subjected to the treatment outlined above. Under favorable conditions almost the entire thallus appears to become involved in sporogenesis, the individual sporangia often consisting of axial filaments from 1 to 2 mm. long and bearing from 6 to 10 well-developed branches. (Pl. 6, A.) The numbers of spores discharged from such extensive sporangial units is naturally very considerable, not infrequently running up to 300 or 400. (Pl. 6, *Bc.*) The individual sporangia are set off from adjacent sporangia, or still undifferentiated hyphae by partitions which may be plane or somewhat curved and are often inserted at the origin of a branch. (Pl. 6, A, D.) A vast number of zoospores may thus be produced in the course of a few hours, estimates made on such material often reaching several hundred thousand. It may be mentioned that the amount of growth observable after the washing away of the nutrient material is quite negligible, the possibility of extensive proliferation of new filaments originating as specialized organs being thus largely precluded. Nor do the sporangial units of such material differ in abundance of branching from the mycelium of the vegetative thallus in an actively growing condition, in spite of the customary characterization of the sporangia of congeneric forms as simple or rarely branching.

A distinction between hypha and sporangium might more plausibly be drawn when older material is used for the production of zoospores. Staling effects are here manifested in the degeneration of the contents of a large proportion of the hyphae. Sporogenesis is never prompt, a period of 48 hours usually elapsing before any considerable discharge occurs. The reason for such delay is evident on examination, when it will be found that the old hyphae are not functioning as sporangia; that these have, in fact, become evacuated, the contents having apparently been utilized in the production of new filaments in the central portions as well as at the margins of the thallus; and that sporogenesis is localized in the newly proliferated filaments, which indeed, show relatively little branching. Manifestly the potentiality of serving as sporangium is not limited to hyphae arising as specialized organs; but is inherent in any vigorous hyphae when given the necessary conditions. It may be remarked that Rothert (*15*) in his study of a congeneric form came to an entirely similar opinion in regard to this phase of reproduction.

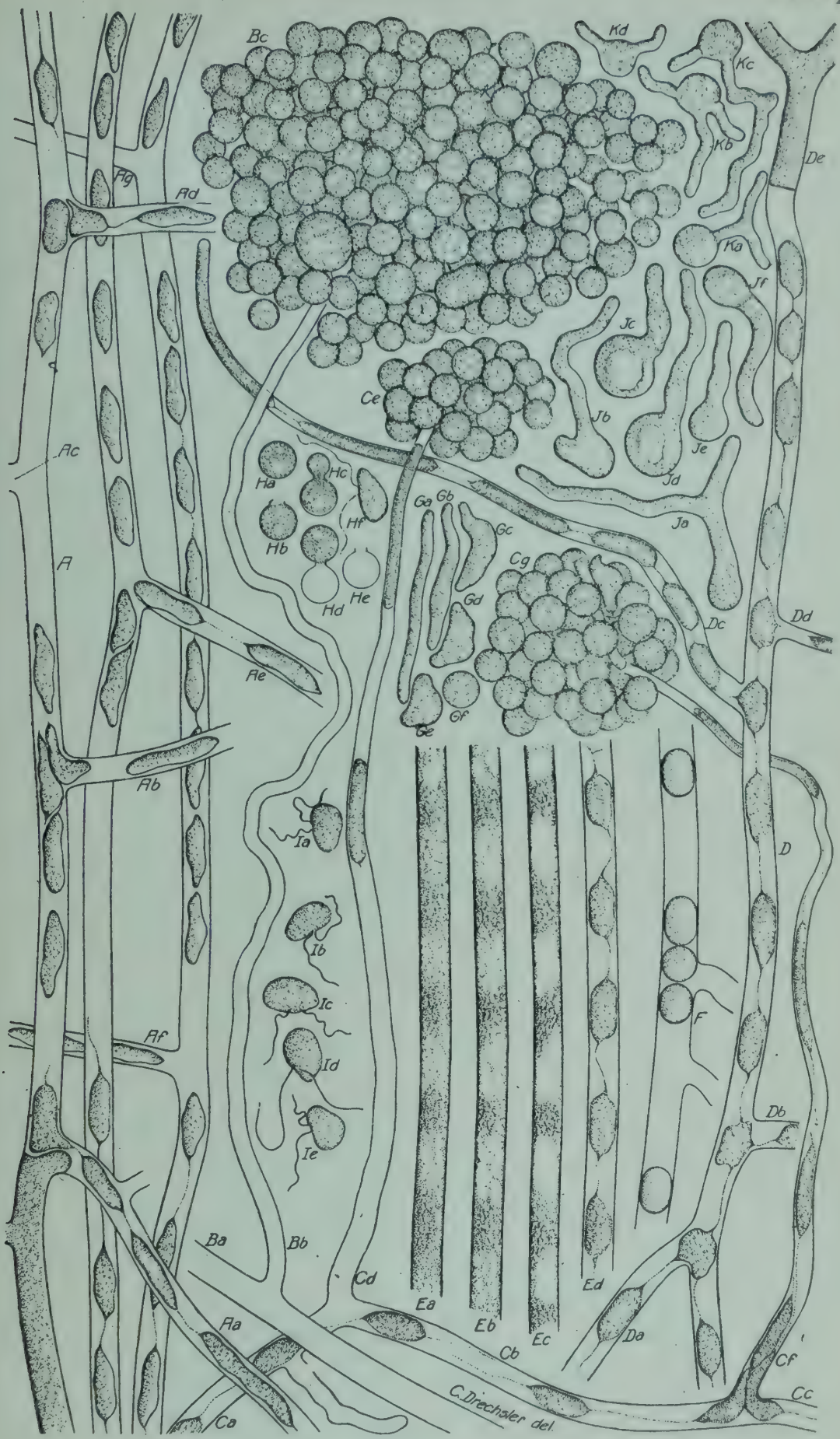
While the hyphae of the pea parasite in liquid culture, as within the host cell, tend toward evenness in diameter, pronounced local irregularities in respect to this dimension not being characteristic of the fungus, the terminal mycelial branches generally exhibit gradual attenuation toward the tip, the apical portion generally measuring 4 μ, or, more rarely, somewhat less. Evacuation of the individual sporangia very regularly takes place through these attenuated branches. As the protoplasmic masses pass from the larger hyphae into the constricted region they become considerably elongated and move at a proportionately increased speed. Thus a cylindrical mass 10 to 12 μ in length, occupying the lumen of a filament 9 μ wide and moving at a speed of about 35 μ a second, on reaching the distal portion of the evacuation hyphae will be found measuring 30 to 50 μ in length and moving more than 100 μ a second. In a few instances a considerable part of the discharge tube was found reduced to a diameter scarcely exceeding 3 μ. Discharge here offered the remarkable spectacle of zoospores drawn out into threadlike bodies, about 2.5 μ in diameter and 70 to 90 μ long, speeding along at the rate of approximately 300 μ per second.

The discharge of the zoospores from the freely branching sporangium presents an interesting complication. At

EXPLANATORY LEGEND FOR PLATE 6

Stages in asexual reproduction of *Aphanomyces euteiches*. ×470

A.—Portion of extensive sporangium drawn in three contiguous sections bearing six successive branches. *ab–g*, evacuation of zoospores arrested after proceeding for some time
B.—*a*, Portion of sporangial unit with aggregation of approximately 350 zoospores
C.—*a* to *c*, Portion of sporangial unit, evacuated at beginning only through branch *cd*, then through both *cd* and *cf*, and finally only through *cf*
D.—Portion of sporangium 15 seconds previous to discharge through branch *dc*
E.—*a* to *d*, Successive stages in the conversion of hyphal contents into zoospores ready for discharge
F.—Zoospores rounded up within sporangial wall
G.—*a* to *f*, Successive stages in rounding up of cylindrical protoplasts, at intervals of 2 seconds
H.—*a* to *f*, Evacuation of encysted zoospore
I.—*a* to *e*, Motile zoospores treated with osmic acid and gentian violet, showing length and insertion of flagella
J.—*a* to *f*, Direct germination of encysted zoospores
K.—*a* to *d*, Germination of zoospores after swarming and second rounding up



(For explanatory legend see p. 306)

each juncture of axial filament and branch two of the elements deliver their contents into the third. Where evacuation is not too rapid, the delivery alternates in a more or less orderly manner, one or several zoospores from one arm being followed by one or several zoospores from the other. When discharge is more rapid, some degree of disorder usually results, a number of zoospores from both sources often being squeezed into the efferent element so compactly that they may momentarily appear as a single protoplasmic mass (pl. 6, *Cf*) which further along in the course of the filament tends to become separated into its components.

As in other members of the genus Aphanomyces, the changes (pl. 6, *Ea*, C), resulting in the division of the mycelial contents into zoospores, leaves the individual protoplasmic masses connected by a delicate strand of tenuous material. (Pl. 6, Ed, D.) This strand can usually be seen without difficulty after evacuation has started, even when it has become further attenuated by lengthening of the intervals between successive zoospores. However, beyond the first juncture of two sporogenous elements the strands uniting the zoospores contributed by each of the elements are pressed against the confining mycelial wall by the interpolated zoospores contributed by the other, with the result that their continuity, if not actually destroyed, becomes at least very difficult to establish. After several junctures have been passed, so that the moving file of zoospores represents, perhaps, more than half a dozen interpolated series, it is certainly not possbile to make out a corresponding number of strands in the intervals. Usually only one or two can be made out in any particular gap, depending upon whether a strand can be demonstrated for one or for both of the two successive zoospores. (Pl. 6, D.) Rothert (*15*) in his study of an unnamed species of Aphanomyces believed that the strand persisted even where none was visible; that the fact of the escaping protoplasts being pointed at the ends indicated clearly enough the presence of a connecting medium capable of exerting a pull. To the traction exerted by the distal zoospores he assigned some importance in accomplishing the evacuation of the proximal ones. In the pea parasite, however, the zoospore while passing through the evacuation hypha are not always pointed at the ends, the anterior end especially being frequently well rounded as if no distorting pull were present.

A curious feature exhibited by the root-rot fungus which does not seem to have been recorded hitherto for any congeneric form is the discharge of the zoospores from a sporangial unit through plural evacuation hyphae. In the extensive units characteristic of vigorous young thalli converted to reproductive purposes, three or even four evacuation tubes have been found; and sometimes two of these may be close enough together that they can be observed simultaneously. Such an instance is represented in Plate 6, C. Evacuation here began through branch *Cd* and had proceeded briskly for about 15 seconds when the tip of branch *Cf* also yielded. For a number of seconds discharge occurred simultaneously with about equal rapidity from both tubes, the element *Ca* supplying tube *Cd*, while *Cf* was supplied from the element *Cc*. Soon discharge through *Cd* came to a standstill, and the element *Ca* contributed its zoospores through the intermediate portion *Cb* into *Cf*, the latter then being fed, as *Cd* had been previously, from both directions. A number of zoospores in the portion *Cb*, that had originally come from *Cc* and seemed bound at the time to emerge through hypha *Cd*, thus reversed their direction and were discharged through branch *Cf*. In other cases evacuation through two tubes was observed to take place simultaneously through both, now through one, now through the other, in repeated and apparently haphazard alternations. The entire process, with its reversals in direction resulting in the discharge of successive zoospores through separate evacuation branches, failed to suggest any considerable effectiveness of visible and possibly invisible connecting strands in determining the course of any individual zoospore.

On emerging from the mouth of the sporangium the spores are cylindrical in shape, straight or slightly curved. (Pl. 6, *Ga*.) Immediately, however, they begin to shorten up, and after passing through increasingly thick allantoid phases (pl. 6, *Gb* to *e*), appear at the end of about 10 seconds as perfectly spherical masses (pl. 6, *Gf*, although a certain proportion of irregular oversized individuals may usually be found. (Pl. 6 *Bc*, *Ce*, *Cg*.) The secretion of a thin peripheral wall follows very shortly. While in certain species of Aphanomyces the quiescent zoospores arrange themselves in a very regular hollow sphere having some considerable degree of coherence, in the form under consideration these bodies show little tendency toward definite orientation and relatively little co-

herence. They seem merely to accumulate promiscuously at the mouth of the sporangium in an irregular lump (pl. 6, *Bc*, *Ce*, and *Cg*), or where smaller numbers are concerned each individual may remain alone.

The development of motile zoospores from these encysted bodies takes place after a period varying in some observed instances from 1½ to 2½ hours. A papilla makes its appearance on the cyst, measuring between one-fourth to one-third the diameter of the latter. (Pl. 6, *Hb*.) At first scarcely discernible, it develops visibly in the course of several minutes, until it appears as a hemispherical protuberance. Suddenly the granular protoplasmic contents begin streaming into the papilla, the tip of which thus becomes inflated into a spherical vesicle. (Pl. 6, *Hc*.) Streaming is completed within approximately 10 seconds, the entire contents having then passed from the cyst into the vesicle. (Pl. 6, *He*.) The contour of the latter is not tangent to that of the empty cyst envelope, as these two structures are separated by a cylindrical isthmus, about 1 μ long, composed of the wall of the papilla. The whole process appears altogether analogous to the discharge of the sporangium of Pythium on a smaller scale, and, as in that genus, the discharged protoplast, after increasing distortional movements, finally develops motility and swims away, not as a number of zoospores, to be sure, but as a single zoospore, somewhat pointed at one end, with two cilia inserted laterally. (Pl. 6, *Hf*.) In material killed with osmic acid and stained with gentian violet, the flagella appear as thread-like structures approximately twice the length of the zoospore, one being somewhat longer than the other but the difference not being pronounced. (Pl. 6, *Ia* to *e*.)

After swimming about for a variable period the zoospore, as in other members of the Saprolegniaceae, finally come to rest and round up. Under favorable conditions they germinate, producing from one to three germ tubes (pl. 6, *Ka* to *d*) capable of extensive development into a mycelium, either in artificial culture or in the tissues of a new host plant.

It may be superfluous to call attention to rather usual irregularities in development, cited frequently in the literature pertaining to related organisms. Among these might be mentioned the failure of the zoospores to escape from the sporangium (pl. 6, F) and their germination within the filament; the production of compound zoospores exhibiting two sets of flagella with erratic ineffectual movement, resulting from the germination of oversized and evidently dienergid cysts; and direct germination, without swarming, of encysted forms by the production of a single rather broad germ tube. (Pl. 6, *Ja* to *f*.)

TAXONOMY OF PARASITE

As suggested in another connection, it is not improbable that the parasite under consideration may have been observed by previous workers investigating the troubles affecting peas. Wittmack (*19*), in Germany, attached the binomial *Pythium sadebeckianum* to a fungus found occurring as oospores in the roots of peas, the presence of which was evidently associated with symptoms having some similarity to those described in this paper. Chiefly because the diameter of oogonia, as given by this author, —32 μ is in excess over those of any species of Pythium commonly attacking higher plants, students of the latter genus have been at a loss as to the identity of the German fungus. Judging from the widespread occurrence of the root rot parasite in diseased pea roots in the United States, it is at least likely that Wittmack, who apparently never observed sporangia in his material, may have assigned his fungus to the wrong genus. For quite similar reasons the same uncertainty attaches to Clinton's (*2*, p. 450–453) provisional identification of oospores observed during recent years in diseased pea roots in Connecticut as the oospores of *Phytophthora cactorum*. The identity of the forms observed by these writers with the fungus discussed in this paper at present can merely be suggested as a very fair possibility.

In this connection it may not be amiss to call attention to the somewhat unusual parasitic character of the rootrot organism when viewed in its taxonomic relation. While the Saprolegniaceae include a number of parasites affecting fish, only a few reports can be found of any members of the family attacking species of higher plants. In 1912 Sawada[5] published a paper containing a detailed study of *Achlya prolifera* (Nees) DeBary, as the cause of a rice seedling

[5] SAWADA, K. INVESTIGATION OF THE PADDY SEEDLING DECAY IN FORMOSA. Formosa Agr. Exp. Sta. Spec. Bul. 3, 84 p., illus. 1912. [In Japanese.] Brief statement of content in English, by W. H. Weston in Ann. Bot. 37: 347–348, 1923. Author's unpublished English résumé revised by W. H. Weston, deposited in Office of Cereal Investigations, Bureau of Plant Industry, Washington, D. C.

decay in Formosa. Nemec (*12*) in 1913 described from Prague as the type of a new genus an interesting fungus causing swellings on the roots of *Salix purpurea* under the binmoia *Jaraia salicis*. It is worthy of note that the fungi in these two instances effected their parasitism under conditions substantially aquatic, the willow plants attacked by *Jaraia salicis* being cultivated at the time in tap water in the greenhouse, while the destruction observed by Sawada presumably occurred while the host was kept flooded.

Within the genus Aphanomyces itself the tendency toward parasitism is moderately pronounced. Two of the eight described species, *A. phycophilus* DeBary and *A. norvegicus* Wille, attack species of Spirogyra and Zygnema (*1; 18*); two other members of the genus according to Coker, *A. stellatus* DeBary and *A. parasiticus* Coker (*3*), attack Achlya, and the same author discusses a variety of *A. laevis* DeBary that was found growing parasitically on diatoms and desmids. In these instances the hosts represent aquatic lower forms attacked under thoroughly aquatic conditions. The only account of a member of the genus, and, indeed, of the family, as far as the writers have been able to determine, attacking one of the higher plants under ordinary terrestrial conditions is contained in the report by Peters (*14*) of a form of Aphanomyces identified by him as *A. laevis*, as one of the three widely prevalent parasites responsible for root blight (Wurzelbrand) of sugar beets in Germany. Peters' publication is of particular interest, although in some pathological features the disease he investigated differs from the trouble affecting the subterranean parts of peas.

In considering the taxonomic disposition of the pea fungus, moreover, *Aphanomyces laevis* represents one of the two species deserving of special attention, the other being *A. helicoides* v. Minden. The remaining six congeneric forms are characterized by the presence on the oogonium of spines or of tuberculate irregularities, whereas the oogonia of the pea parasite, like those of the two species designated, are entirely smooth on the exterior. According to its author (*11*), *A. helicoides* is very similar to *A. laevis*, being distinguished chiefly by a strong tendency in the antheridial branches to wind about the oogonium, or about ordinary hyphae, or even about other antheridial branches, in close helicoid turns. Certain of Kasanowsky's drawings (*10:* Taf. X, fig. 1) represent some such condition, making it seem probable that this investigator was dealing with a type more nearly resembling the Swiss form than the one originally described by DeBary. Coker seems inclined to support Von Minden's own doubts concerning the validity of the helicoid habit as a specific distinction on the ground that a strong tendency toward such habit was exhibited by the typical form of *A. laevis*. Whatever disposition may finally be taken with reference to *A. helicoides* is, however, of minor concern here, for, as has been previously pointed out, no distinct tendency toward spiral growth has ever been found expressed in any of the cultures of the pea organism the writers have studied, although the branches bearing the sexual organs may be more or less involved after a more promiscuous fashion.

When the organism responsible for root rot of peas is compared with the typical aquatic form of *Aphanomyces laevis*, as revealed in the literature, a considerable measure of agreement becomes evident. The dimensions and interrelations of oogonium and antheridium and of the branches supporting them, as well as the dimensions of the oospores, correspond quite well in the two plants. A fair, even if not altogether perfect, agreement is evident in regard to size of zoospore and diameter of mycelium. The potentiality of the entire thallus to serve reproductive purposes by division into extensive branching portions functioning as individual sporangia; the discharge of the latter through one or several hyphae tapering regularly toward the tip to a diameter inferior to the diameter of the mycelium generally; the ready germination of the oospores, and that without resting period or application of special treatment—these features point to a distinction between the root parasite and the aquatic form, although it remains doubtful to what extent absence of their mention in the literature pertaining to the latter form is attributable to actual difference in its behavior or to shortcomings in our knowledge concerning it.

With regard to another character—namely, the thickness of the oogonial wall—it becomes almost impossible to entertain similar doubts. The accounts of *Aphanomyces laevis* given by DeBary, Humphrey (*5*), Kasanowsky, and Coker contain no reference to this feature, yet it is altogether unlikely that any of these investigators could have seen a structure of such extraordinary thickness without in some way referring to it. Indeed, on the contrary, Coker in his definition of the genus

Aphanomyces, recognizes a thin unpitted oogonial wall as a generic characteristic, presumably common to all species; and all of the authors mentioned figure the oogonial envelope of *A. laevis* as a thin membrane, not to be compared with even the least indurated of the homologous structures belonging to the parasite on pea roots.

In this connection it may be mentioned that the writers have cultivated an aquatic species of Aphanomyces with smooth oogonia, obtained through the courtesy of J. A. Lounsbury, who isolated it from water of Lake Mendota, where apparently it occurs in some abundance. This fungus, which as far as could be determined answers to the description of *A. laevis*, very obviously is not identical with the parasite affecting peas, developing a heavy matted, submerged growth on various agar media entirely unlike the arachnoid growth characteristic of the terrestrial organism. The oogonia are produced much more sparingly and always with the thin membranous wall made familiar by the figures of various writers. As the different isolations made from diseased peas compared with one another show unusual uniformity with reference to morphological characteristics, as well with reference to rapidity and character of growth on a variety of substrata, their incorporation in the same species with a form so evidently dissimilar as the aquatic plant could scarcely contribute to taxonomic clarity.

The identity of Peters' beet parasite remains problematical. This author undoubtedly was right in concluding that the measurements of his fungus, while somewhat different from those of *Aphanomyces laevis*, did not, in themselves, justify its recognition as a species distinct from the latter. It is interesting to note that he observed very marked variability in thickness of both oogonial and oospore walls, and as the thickness of these structures fluctuated together he believed that the fluctuations were contingent on the same environmental conditions affecting both. Thus in material containing oogonia so thin-walled that they were found collapsed, the oospores borne in them were provided with walls *only* 3 μ thick; while in other material oogonia with walls 1.5 μ thick contained oospores of which the envelope measured 5 to 6 μ in thickness. If the measurements of the oospore wall as given represent measurements of normal material, Peters' fungus would undoubtedly constitute a species different from any species of Aphanomyces hitherto described, as well as from the one described in this paper. However, grounds for suspecting that this is not the case are not wanting. In the pea parasite, for example, degenerate conditions of the sexual apparatus are far from rare even on the most favorable substratum like hard corn-meal agar; when less favorable media are used (potato or carrot decoction with 1.5 per cent agar, and also pea decoction in the absence of solid particles) degenerate conditions represent the overwhelming rule. The contents of the oogonium may become lumpy and degenerate before an oosphere is formed, or an oosphere may be formed but degenerate without forming a wall, or a wall may be formed but the contents degenerate instead of developing the normal structure. In any case swelling of the confining membrane is a regular concomitant of protoplasmic degeneration, whether this occur in oogonium or oospore, and in the absence of normal material such pathological modification might be mistaken for normal thickening. In view of these circumstances, it is hardly possible to decide definitely as to the morphological relationship of the two congeneric forms, both terrestrial in habitat and parasitic on a phanerogamic host.

It may also be stated that in the study of the pea organism structures involved in degenerative changes were not given weight; that the peculiarities of the oogonial wall submitted as normal were observed regularly in nature, as well as in culture, in individual female organs containing oospores provided with a wall of moderately and practically unvarying thickness containing a large, homogeneous, slightly eccentric ("subcentric" in the phraseology used by Coker) structure, surrounded by concentrically arranged granular-appearing bodies. The parasite, it is believed, may best be regarded as a new species. Because of the distinctive character of the oogonial envelope, the specific name *euteiches* is suggested.

TECHNICAL DESCRIPTION

APHANOMYCES EUTEICHES Drechsler n. sp.

Parasitic on the subterranean parts of cultivated peas (*Pisum sativum* L.), causing a destructive stem and rootrot capable of affecting the growing host at all ages.

Hyphae hyaline, branching at moderate intervals (20 to 150 μ) at angles approaching a right angle; 4 to 10 μ in diameter, the individual filaments not

abruptly varying in width; occurring in nature within cortical cells of host, in nutrient solutions as extensive nebulous translucent mycelia.

Sporangia in artificial culture arising by conversion of extensive portions of vegetative mycelium delimited by one or more septa; often including many ramifications; discharging through one or several (up to four) tapering branches, the distal portions of which measure usually approximately 4 μ.

Zoospores cylindrical, in escaping from evacuation branches becoming attenuated to vermiform bodies, usually 3.5 μ in diameter by 30 to 50 μ in length; forming spherical cysts at mouth of sporangium, measuring usually 8 to 11 μ in diameter, rarely up to 16 μ; diplanetic, the empty spherical wall being distinguished by a protruding evacuation tube 1 μ long by 2.5 to 3 μ in diameter.

Oogonium generally, if not always, terminal on a short lateral branch, from which it is delimited by a partition sometimes present as a simple septum, at other times as a columella-like structure protruding into the oogonial cavity; subspherical, measuring usually 25 to 35 μ in diameter; when mature exhibiting a heavy peripheral wall with smooth outer contour and sinuous inner contour, hence of irregular thickness, this dimension varying between 1 to 5 μ, generally between 1 to 2.5 μ.

Antheridia typically of diclinous origin, borne on a stalk frequently involved with the oogonial stalk, and often branching once or several times; measuring 8 to 10 μ in diameter by 15 to 18 μ in length, or when considerably larger often more conspicuously arched, somewhat lobulate, and becoming compound by the insertion of transverse septa.

Oospores subspherical or more rarely ellipsoidal owing to intruding columella-like septum; 18 to 25 μ (generally 20 to 23 μ) in diameter; provided with a wall of uniform thickness, this dimension varying between 1.2 and 1.8 μ (generally 1.5 μ); slightly eccentric in internal structure ("subcentric"); germinating without protracted resting period either directly by 1 to 3 germ hyphae or by production of a single unbranched sporangial filament usually 200 to 350 μ in length, in the latter event producing generally 13 to 18 zoospores, approximately half of which are delimited within oospore wall.

Collected repeatedly in diseased peas in Maryland, New York, Ohio, Indiana, Michigan, Illinois, Wisconsin, Montana, Idaho, Utah, and California.

PHYSIOLOGY OF PARASITE

GROWTH OF THE FUNGUS ON CULTURE MEDIA

The fungus has been grown on several of the common culture media, on all of which it produces more or less of a sparse white surface growth with little aerial mycelium. A few imperfectly formed oospores are usually found in older cultures on most media. However, on corn-meal agar, while the mycelial growth is rather less than on most substrata tried, there is a moderately abundant production of oospores which are capable of germination soon after they are formed. For this reason cultures on this substratum retain their vitality indefinitely, while those on other media soon perish. Semisolid media, such as cornmeal or oatmeal with varying amounts of water added also give good growth, but the oospores are less perfectly formed as the medium becomes more moist. The most useful liquid medium that has been found for the production of mycelium and sporangia is a pea decoction made by adding 10 to 20 peas, preferably of a wrinkled variety, to about 75 cc. of water in a flask. This liquid remains clear after sterilization in the autoclave, and mycelium growing in it can readily be washed free from all nutrient material. The fungus grows as a submerged tuft in this medium until it reaches the surface, over which it spreads as a mat. In about 7 to 10 days at room temperature oospores begin to form, and soon after most of the mycelium is found empty and dead.

EFFECT OF TEMPERATURE UPON THE DEVELOPMENT OF THE FUNGUS

Growth of mycelium.—The fact that this disease develops early in the spring indicates that the fungus must be able to thrive at comparatively low soil temperatures. In an experimental determination of the thermal limits within which the fungus will grow a semiliquid medium, composed of 1 part by weight of corn meal with 12 parts of water, was used. The growth of the fungus in the surface of this gelatinous material is so inconspicuous that small increments of growth can not be measured accurately, and while the limits of growth were determined, the optimum could only be roughly estimated. In cultures held at a series of constant temperatures in incubators vigorous growth occurred at 34° C., but no growth was observed at 37°. At the lower end of the series no growth was observed after six days at 8° to 10°,

while a growth of 1.5 cm. occurred at 9° to 11° in the same time. The optimum temperature for mycelial growth appeared to be between 15° and 34°.

FORMATION OF ZOOSPORES.—The temperatures at which zoospores were discharged from sporangia and became motile was determined as follows: A culture upon oatmeal mush six days old was washed as free as possible from its substratum, cut into fragments, and placed in sterile water in watch glasses which were distributed in incubators at a series of temperatures. The results are presented in Table I.

drops of sterile water to which spore-bearing mycelium with as little substratum as possible has been transferred. Germination has been secured at temperatures from 6.5° to 31° C. Germination with the production of zoospores occurs in 24 to 48 hours at 14° to 28°, a range that may be regarded as an optimum. Germination with the production of a few zoospores has been secured at a temperature as low as 9° to 10° in four days, while germ tubes which did not discharge zoospores developed in the same time at 6° to 7°. Thus the oospores germinate in practically the

TABLE I.—*The effect of temperature upon the formation and motility of zoospores of Aphanomyces euteiches and the germination of zoospores*

Temperature °C.	Time in hours					
	18	25	42	49	73	97
33–35		Groups.	Groups.	Disintegrating.		
21–22	Groups.	Groups.	Groups. Zoospores.	Inactive.		
19.5–21	Groups.	Groups. Zoospores.	Groups. Zoospores.	Inactive.		
15–16	Groups.	Groups. Zoospores.	Groups. Zoospores.	Groups. Zoospores.	Groups. Zoospores.	Inactive.
13–14		Groups.	Groups. Zoospores.	Groups. Zoospores.	Groups. Zoospores.	Inactive.
9–11			Groups.	Groups. Zoospores.	Groups. Zoospores.	Zoospores. Groups.
8–9.5						
5–6.5						

NOTE.—The word "Groups" indicates the presence of groups of discharged encysted zoospores at the time of observation, and "zoospores" the presence of motile conditions.

GERMINATION OF OOSPORES.—The determination of the effect of temperature upon the germination of oospores has been rendered difficult by reason of the fact that no dependable method has been developed whereby a quantity of mature oospores can be produced at a given time. Oospores from the roots of peas have not been seen to germinate. Frequently cultures on corn-meal agar three weeks old will germinate readily when placed in water, but sometimes only one culture among many made at the same time will respond in this way. Spores on the scant aerial mycelium which can be separated from the substratum usually germinate sparsely, while spores on or in the substratum often begin germination as soon as mature. In spite of the difficulties which have arisen from these irregularities in behavior, fragmentary records have been made which appear to delimit closely the range within which germination occurs and the optimum. Germination has been studied in hanging

entire range of temperature at which vegetative activity occurs, and the discharge of zoospores from the sporanges produced by the germination is affected by temperature in exactly the same manner as in the previous experiments where sporanges derived more remotely from oospores were used.

PATHOGENICITY

PENETRATION

A study of lesions in early stages occurring in the field or in greenhouse inoculations rarely reveals the first penetration of the fungus into tissue. In roots entry is accomplished without discoloration or visible indication of the fact, and later the entering mycelium, devoid of contents, is practically invisible. In the base of the stem a yellow discoloration in a few epidermal cells often indicates a point of entry. The invasion of the base of the stem is usually accomplished by the advance of the fungus upward from the root

rather than from direct entry. When peas are sterilized superficially and grown under sterile conditions in agar upon which the fungus is also growing, penetration of roots can then be found readily in thin razor sections of the root surface. The mycelium passing along the root surface turns inward at the junction of two epidermal cells and passes readily into one of these cells, whence it advances into an adjoining cell or into underlying tissue. (Pl. 3, B.) The point of entry and passage from one cell to another is not marked by any conspicuous constriction of the fungus strand. Invaded tissue softens immediately even in the absence of bacteria, indicating perhaps the production of an enzyme which softens cell walls and aids in fungal penetration.

RELATION OF SOIL TEMPERATURE AND MOISTURE TO THE DEVELOPMENT OF THE DISEASE

EXPERIMENTAL STUDIES

Among the many diseases of roots of plants few show in the field a greater degree of apparently erratic irregularity than the one under consideration. Even in fields which are very severely infested the disease is usually much worse in spots, and at its first appearance it occurs in irregular areas which are frequently coincident with the more moist soil, but at other times are not clearly related to any obvious soil differences. It appears that the occurrence of this disease is greatly influenced by environmental conditions. In its seasonal development temperature is undoubtedly a limiting factor, and in its local occurrence the field evidence suggests soil moisture as the more important limiting factor. In order to gain exact experimental evidence of the relations of these factors to the development of the disease, several series of plantings were made in the Wisconsin soil temperature tanks where both temperature and moisture were controlled within narrow limits. Two of these series will be reported in detail.

The soil chosen was a sandy loam from a field in which peas had never been grown, and as a further precaution against the presence of root-destroying fungi it was treated with formaldehyde a month before use and subsequently thoroughly dried until no trace of this sterilizing agent was detected. The soil was inoculated one week before planting by mixing thoroughly with small fragments of roll cultures of an isolation of this fungus which had shown very active parasitism in previous tests. The viability of the oospores in these cultures had also been previously demonstrated. The inoculated soil was packed in 6-inch cans, near the bottom of which an irrigation apparatus was arranged to permit the addition of water without flooding the surface of the soil. On November 18, 15 Alaska peas were planted at the depth of about 2½ inches in each can. Six cans were placed in each of a series of tanks, which were maintained at 12°, 18°, 24°, and 30° C., respectively. The moisture present in the soil at planting was 15 per cent of its dry weight, or about 35 per cent of the moisture-holding capacity of the soil, previously determined to be 42 per cent of its dry weight. Two of the cans in each tank were maintained at the original soil moisture, while in two others the water was increased to 60 per cent of the moisture-holding capacity of the soil, and in the remaining two cans it was raised to 80 per cent of the moisture-holding capacity.

Inoculation in this instance was so effective that soon after they had emerged from the ground a few of the plants decayed and wilted down, whereupon they were removed. At the conclusion of the series, on December 18, the remaining plants were removed from the soil, and record taken of those showing decay of the base of the stem and of the roots. (See Table II.)

From these data it appears that the fungus was restrained little if any in its parasitic activity toward peas at 30° C., which is within 3° of the upper limit of soil temperature at which peas can be induced to make an approximately normal growth. In soil with the highest moisture content the progress of the disease does not appear to be favored as much as might be expected. Since some degree of infection took place even at the lowest soil temperature maintained here, and the activity of the parasite at the lower range of temperature is of greater interest, a new series was started December 19, with the purpose of exploring more thoroughly the range of temperature which occurs in the field at the time when the disease normally appears.

In the second series the soil used before was mixed, dried somewhat, and placed in cans. The moisture in this soil was found to be 36 per cent of its moisture-holding capacity, and adjustments were made to 60 and 80 per cent to correspond with the earlier series. (See Table III.)

TABLE II.—*The pathogenicity of Aphanomyces euteiches to Alaska peas at the series of soil temperatures and in soil at the three conditions of soil moisture indicated (series of November 18, 1923)*

Temperature in °C	12°			18°			24°			30°		
Per cent moisture-holding capacity of soil	35	60	80	35	60	80	35	60	80	35	60	80
Number of plants Dec. 1	25	27	25	27	26	26	25	25	24	28	26	27
Number of plants wilting and removed at date indicated:												
Dec. 1								1	2			3
Dec. 2								2	4			2
Dec. 6							2	1	7		7	6
Dec. 8					1	2		3	1		7	2
Dec. 13					1	2	8	5	4		4	2
Dec. 17				3	2	1	5	2			2	1
Total number of dead plants				3	4	5	15	14	18	(a)	20	16
Number of plants with decayed stem bases Dec. 18	3	9	3	10	19	11	10	4	(b)	1	3	8
Total number of plants infected	3	9	3	13	23	16	25	18		1	23	24
Healthy plants, Dec. 18	25	21	25	15	5	8	0	5	(b)	12	4	1

[a] One of the two cans at this temperature and moisture developed a leak and was discarded.
[b] Data missing.

TABLE III.—*The pathogenicity of Aphanomyces euteiches to Alaska peas at the series of soil temperatures and at the three conditions of soil moisture indicated (series of December 19, 1922)*

Temperature in ° C	10°			12°			15°			18°			21°			24°		
Per cent moisture-holding capacity of soil	36	60	80	36	60	80	36	60	80	36	60	80	36	60	80	36	60	80
Number of plants Jan. 5							27	36	32	34	34	28	38	24	32	39	31	28
Number of plants wilting and removed at date indicated:																		
Jan. 2												1			1		1	3
Jan. 5														2	1			
Jan. 9									1			5		4	3	4	1	13
Jan. 12									2		1	1				6		3
Jan. 19									3	5		4		1	3	7	2	4
Total number of plants killed									6	5	1	11		7	8	17	4	23
Number of plants diseased Jan. 30		1	1	2	0	2	17	8	10	22	19	6	10	5	17	12	7	5
Total number of plants infected		1	1	2	0	2	17	8	16	27	20	17	10	12	25	29	11	28
Healthy plants Jan. 30		29	20	30	32	19	14	25	16	6	13	10	24	13	8	7	17	0

The soil from this experiment was used for a third series in the temperature tanks, in which the method was modified for the purpose of learning whether infection occurring later in the development of the plant would produce less wilting than early infection. The soil was dried until it contained 30 per cent of its moisture-holding capacity, mixed thoroughly, and placed in cans. Peas were planted on February 10, and the entire series was kept at a soil temperature of 12° C. until February 24, when most of the plants had emerged, though not all had assumed an erect position. Temperature was adjusted to 15°, 18°, 21°, and 27°, and water was added to bring the soil up to 60 per cent of its moisture-holding capacity. New inoculation of these plants was then made by pouring a suspension of zoospores around the bases of all plants, and the surface soil was kept moist for the three following days. In five days a few plants at 27° showed infection at the surface of the soil, from which they died later; but there was so little infection at the lower temperatures that the results of this series are not

presented in detail. The paucity of infection in this experiment is not easily explained.

The data presented from the two previous series indicate clearly that though occasional infection may take place through practically the entire range of temperature at which peas will grow, infection is not abundant nor does invasion proceed rapidly at a temperature below 15° C. The optimum temperature for infection is between 15° and 30°. Within this range infection appears to be approximately uniformly abundant, but at the lower temperatures there is a retardation in rate of progress of the fungus through the plant that is even greater than the retarding effect of temperature upon root growth as it occurs under the conditions of experiment. It is probable, however, that in the field in the spring with longer days root growth is more rapid at lower temperatures than under the conditions of experiment, and if that is the case the apparent retarding effect of low temperature upon the progress of the disease in the field will be greater than is shown here.

The effect of soil moisture upon infection in these experiments was not as great as anticipated. In fact, they do not give any decisive indication that soil moisture within the limits used in these experiments is a factor at all in determining the amount of infection. Although infected plants in the more moist soil uniformly begin to die a little earlier than those in drier soil, the number is in most cases approximately equal.

Further consideration of these experimental data may serve to bring out more clearly their limitations when an attempt is made to interpret field experience in their light. In these experiments it may be assumed that an approximately equal amount of viable fungus mycelium was brought in contact with the root systems of plants. The number of infections obtained is an indication of the ability of the fungus to infect under these conditions. It is possible that in the field soil moisture exerts an important effect, not only upon infection, but upon the survival of the fungus from year to year. The favorable effect of wet soil upon the disease may be due in large part, if not wholly, to the favorable environment which moisture provides for the fungus in its resting condition as oospores, or in a possible saprophytic life in the soil. Such an effect of soil moisture increasing the active vegetative growth of the fungus in the field may account for the association of disease with wet soil, rather than any effect of moisture upon the penetration of the plant by the parasite.

These experimental data should be compared carefully with observations of the development of the disease in the field on infested land to determine whether the inferences which may be drawn from the experiments are supported by practical experience. Unfortunately, there is but a limited amount of exact field observation which will serve for comparison. The development of the disease in experimental plats at Madison, Wis., on ground thoroughly infested with the fungus has been followed more closely than elsewhere. Planting in this plat was made in 1922 and 1923 on the same date, April 28. In 1923, the roots from these plants remained free from any trace of disease until May 30, when a very few rootlets showing typical decay due to Aphanomyces were found. On June 5 the entire root systems of these plants had begun to decay from a great number of infections distributed from the surface of the ground to the deepest roots. In this brief space of time abundant oogonia and a few mature oospores were formed.

This sudden appearance of disease followed promptly after the first favorable period of temperature and moisture that had occurred since the crop was planted. (Fig. 1.) The month of May of that year was exceedingly dry. The only rains sufficient to wet the soil for a brief period had fallen on May 15, 19, and June 2. The first rain occurred when the plants were emerging from the ground, and was followed by cold weather, the soil temperature [6] at a depth of 2 inches falling at night as low as 1° to 7° C. The second rain on the 19th was followed by weather hardly warmer, the mean soil temperature for the next five days ranging from 9° to 13.3°, a temperature at which oospores would germinate very slowly

The temperature rose very slowly after this date until June 2, when a heavy thunder storm gave a precipitation of 0.92 inch, wetting the soil in the plats to a depth of about 5 inches. After this day the minimum soil temperature did not fall below 15° C., and the mean daily soil temperature rose in the following five days from 17.5° to 20.5°. This coincidence of moisture with high soil temperature furnished optimum conditions for the

[6] The writer is indebted to J. G. Dickson for the use of soil temperature records quoted here.

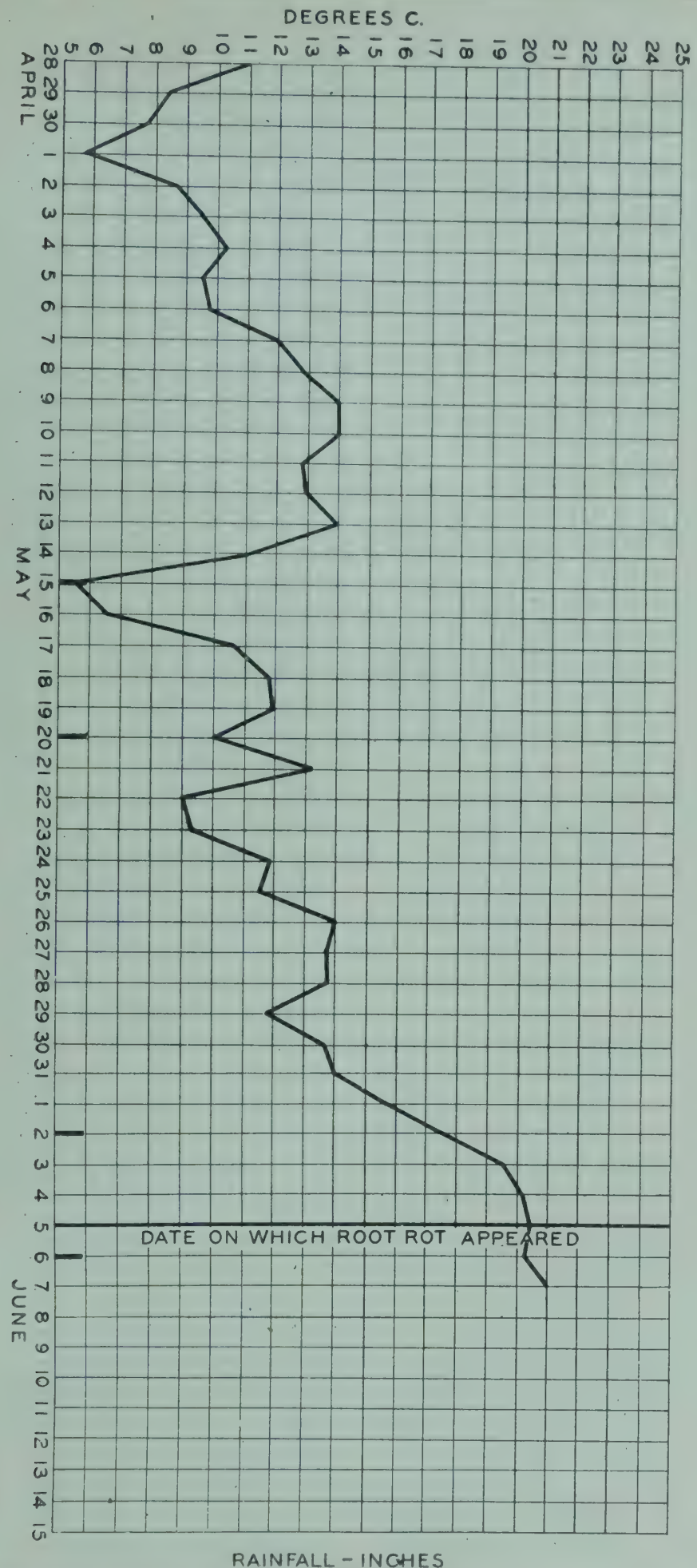


FIG. 1.—Average daily soil temperature in experimental plats at a depth of 2 inches and precipitation at Madison, Wis., from April 28 to June 15, 1923.

development of the disease which appeared with such remarkable suddenness. In fact, the period of time elapsing between the rain and such extensive invasion of the plant was so brief that it seems unlikely that the rain started the fungus to active vegetative growth from oospores, but rather that it had achieved some development during the period of dry weather. A heavy rain on June 6 with continued high soil temperature maintained favorable conditions for the development of the disease for a brief time until it was checked by returning drought, which lasted until the end of the growing period of the crop. It may be added that the shower of June 2 was local, not extending to the entire pea-growing region of the State, and that the general rain of June 6 was in many places the only precipitation during the entire season which wet the soil for even a brief period after temperature permitted infection of plants. Thus the fact that conditions suitable for infection did not occur until comparatively late in the season and were maintained for so brief a period reduced the amount of infection and injury from this disease to a very small amount that year.

In 1922 the onset of the disease was not as sudden or as severe. Some infected plants were found first on May 29, and thorough decay of entire root systems had occurred on June 2. Unfortunately no soil-temperature records were kept at Madison during the month of May of this year which can be compared with the records of 1923 previously cited. However, the Madison Monthly Meteorological Summary shows that the warmest weather of the month occurred May 9 to 12 during a dry period. Precipitation of over 2 inches occurred May 23 to 26, followed by six days with an average mean daily air temperature of 17.7° C., an average which is over 3° higher than of the six days after the rain of May 19, 1923, when no infection occurred. If this increase in air temperature was accompanied by a corresponding increase in soil temperature, a safe assumption since the weather record showed that they were days of almost uninterrupted sunshine, it would be just sufficient to provide a favorable temperature for infection, as determined in controlled experiments. It will, however, be necessary to accumulate many exact observations of the date at which disease appears and records of soil temperature and rainfall preceding the appearance of disease before it will be possible to predict accurately from weather records alone when the disease may be expected to appear; but it seems not unlikely that the time will come when such prediction can be made with precision.

RELATION OF THE PARASITE TO THE HOST

METHODS AND RESULTS OF EXPERIMENTAL INOCULATION IN THE FIELD

It will not be a matter of surprise that a fungus with a mycelium which is converted into perishable zoospores very readily and which forms oospores apparently under such restricted environmental conditions is not only limited in its ability to persist in soils, but, when present, is not always in active condition ready to invade plants. To secure such early and complete ruin of plants from inoculations with pure cultures, as often occurs in the fields, is not always an easy feat. The first difficulty which was encountered, though it appears to have been quite exceptional, deserves passing note. The first culture of this species, which was isolated, proved in the preliminary inoculation experiments to have so slight a degree of pathogenicity that the senior writer was misled and was delayed in recognizing the true cause of this disease. There is no apparent reason for the lack of pathogenicity of this isolation, and none of the seven later isolations which have been used in inoculation experiments have been thus deficient, though there appears to be a slight difference in virulence between some of them.

For greenhouse experiments inoculation of the soil by mixture of fragments of culture on corn-meal agar which have many mature oospores is the most satisfactory. Although such soil does not often give more than 50 per cent severe infection when first used, on a second planting will usually give better results, especially if care is taken to prevent drying out at any time. The use of zoospores in soil, either at the time of planting or poured on the soil when the plants have emerged, is uncertain. When plants are grown in sand, however, zoospores poured around young plants have given very thorough infection, provided great care is taken to keep the sand saturated for a time after inoculation is made.

Field inoculations have been made only at Madison, Wis., in 1923, a year so dry that it was everywhere unfavorable for the development of the disease. Inoculum was prepared in

several forms—earth previously inoculated with cultures in greenhouse experiments, cultures containing oospores planted with the seed, and zoospores applied at planting and at various times later. It seems inadvisable to present detailed results obtained thus in a single unfavorable season. Suffice it to say that some infection was obtained by all methods, but that the effectiveness of the method seemed to depend more upon the rainfall and temperature at and immediately after inoculation than upon the method. Zoospores poured upon the soil about Alaska peas breaking ground just before a rain on May 17 gave as high as 86 per cent infection, and when the soil was removed, exposing about 2 inches of the stem before the spores were distributed, every plant became infected, as evidenced by examination of the roots. Conspicuous decay following this infection of roots did not begin in any case, however, until the disease developed in plants growing on naturally infested soil. This simultaneous development of disease in plats whether infested naturally or artifically suggests that soil temperature controlled this development regardless of the time and manner of infection. All diseased plants in plats inoculated artifically grew to normal maturity and showed no conspicuous evidence of reduced vigor.

In contrast with these results of artificial inoculation were some data on the effects of natural inoculation in a similar type of soil not far removed where peas had been grown repeatedly for 7 years. Double rows of peas one rod long were planted on the infested soil, and extensions of these rows upon ground unused for peas were likewise planted with 400 peas to each rod. Unfortunately the disease had spread somewhat to the new land, so that many roots became infected, especially those of the susceptible variety, Surprize. However, the peas on the infested land showed early in the season all the symptoms of severe root rot, whereas those on the new land showed little indication of disease except at the ends of the rows adjoining diseased ground. Careful examination of plants in these plats during the season failed to reveal any other disease than that caused by Aphanomyces. The yields of some of these rows are given in Table IV.

The striking contrast between the results of natural infection and of artificial inoculation in the field plats is certainly not due to actual killing of the plants and demands explanation.

TABLE IV.—*Yields of dry peas in one rod of double row of peas upon soil heavily infested with Aphanomyces euteiches compared with yields upon adjoining ground where peas had not been grown previously. Planting was made April 28, 1923; 400 peas were planted to each rod of double row*

Variety	Infested land		New land	
	Number of plants growing	Yield	Number of plants growing	Yield
		Grams		*Grams*
Alaska	333	250	367	420
Do	369	210	378	393
Surprise	237	60	267	167
Do	297	71	292	210
Alaska	364	172	346	328
Do	360	125	358	318

Very careful and frequent examinations were made of the roots in all plantings. From these examinations it was found that the number of infected roots of the plants inoculated artificially were few and restricted to the region in which the inoculum had been applied, whether above, below, or near the seed. Under the conditions obtaining during this season, even the complete destruction of the cortex of the base of the stem rarely injured a plant greatly, provided most of the roots remained free from invasion. Although in artificial inoculations trenches in which peas were planted were drenched with zoospore suspension at the time of planting, infections were few and only near the seed. No method of inoculation after the plants had started growth seems to have filled the soil very thoroughly with the parasite in such condition that it was ready to invade the plant at a large number of points at once when favorable conditions for such infection occurred. It appears from field observation described elsewhere, as well as from these experiments, that this disease can produce conspicuous injury to top growth only when plants are either infected under favorable conditions for the development of the parasite when the plants are small or when they suffer a large number of infections at a later stage of growth. In these artificial inoculations the fungus was not distributed deeply and thoroughly enough in the soil to make possible a great number of infections when the brief favorable period for infection arrived; so brief was the favorable period that inoculations of young plants made when it

arrived did not have time to give a great number of infections before dry weather checked the growth of the fungus and prevented further infections and perhaps checked the progress of the fungus through the host tissue.

DISSEMINATION OF THE FUNGUS

The character of the fungus described in the previous pages points clearly to certain obvious methods whereby it may be distributed. Any transfer of soil from diseased fields will carry the parasite with it. In localities where soil from old fields is used to inoculate new fields with the bacteria which produce the beneficial nodules on the roots the parasite also will be carried if it is present. An excellent example of the harm which may be brought about in this way was seen in 1922 in Maryland. Soil from an old pea field in which the present crop was withering before maturity because of this root rot had been used to inoculate several neighboring fields used for peas for the first time. In every one of these fields thus inoculated individual plants scattered uniformly were dying, and others were infected, not enough in all to reduce the yield of this first crop appreciably, but enough to infest the soil so thoroughly that the failure of future crops was assured. Since transfer of soil from old fields to new may carry any of the known pea diseases which happen to be present, this practice is so often the cause of ruin to crops which are grown two or three years after the fields have thus been inoculated with disease that it appears to produce on the whole much more harm than benefit.

The flow of surface water over adjacent fields is an excellent conveyor of the fungus to new ground. The course which floods have taken across fields is sometimes indicated by areas of blighted peas. Irrigation water may be an excellent conveyor of the parasite. When the soil of an infested field is light and blown by wind, it may be a source of infestation for a large territory. In this manner a few centers of infestation are believed by R. E. Vaughan, who is familiar with the territory, to have ruined the entire area of the Truax Prairies in Eau Claire County, Wis., for pea growing. In the spring of 1922 some early plantings of peas were visited at Rochelle, Ill., when a large part of the land surface was being prepared for planting. A violent wind was drying out the surface soil so rapidly and picking up so much dust from it that the whole level country seemed covered with a fog clinging close to the ground. The distribution of spores with soil particles from a few infested fields in this locality under such conditions may account for the occasional diseased plants which are sometimes found when peas are grown for the first time in this vicinity, and the rather rapid subsequent increase of infestation.

There is a widespread opinion among growers that this disease is often introduced into new fields by the use of infected pea seed grown in diseased fields. This opinion, however, does not appear to be justified. Since the fungus does not enter the plant above the ground, it never reaches the seed and could only become attached to the seed in particles of dust from the soil. Even though this might conceivably happen, it appears to be a remote possibility, and, in any case, very few of the spores which could withstand drying would be present, so few that they would almost certainly escape detection by any experimental method that could be used and, therefore, so few that the disease which they would produce would not become conspicuous until several successive crops of peas have been grown. Although it is impossible to say that new infestation never takes place in this manner it appears to be at least a rare occurrence.

CONTROL MEASURES

CROP ROTATION

Although from early times the tradition of pea growing warns of failure which will attend the planting of peas on the same ground repeatedly, and English experience has developed a rotation of five or six years' duration as an insurance against disease, there are, on the other hand, so many advantages in intensive culture of the crop for the commercial purposes for which it is chiefly grown in the United States that rotation has usually been adopted only when the failure of the crop made this course necessary. A long rotation has been found to have a number of disadvantages of more or less importance. In some localities experience has abundantly shown that the second crop of peas on new land thrives better than the first, and the third may produce a better crop than either of the preceding. Peas grown for canning can often be produced more cheaply in a limited area close to the canning plant than in the much larger territory required by a long rotation. In the pea-seed-growing dis-

tricts of Montana, Idaho, and other Western States, this crop is sometimes more profitable than others, and since the suitable land area is limited the temptation to grow peas repeatedly is strong. Under the pressure of these economic reasons which favor the intensive culture of peas, many questions regarding rotation have become of vital interest to growers—how long peas may be grown before rotation is necessary, how short the rotation can be made with safety, and whether perchance new pea-growing districts may be found from which disease can be excluded or in which it will not develop.

Rotation as a control measure in combating pea diseases is generally discussed, not as a remedy for one of the many diseases of foliage and of roots, but as a control measure suited to secure relief from all of them in so far as they occur in the locality. Therefore, even though it is properly outside the limits of this paper to discuss more than the rotation that is needed to secure protection from the root rot caused by *Aphanomyces euteiches*, it will be futile to recommend a rotation which is suitable to protect from this disease alone. In the following pages this matter has been kept in mind, and, although it will be shown that a suitable rotation for peas is a local matter which must be determined with reference to the soil and climatic conditions existing there, it may be said that it appears generally true that any rotation which will control this form of rootrot will be adequate to hold other diseases sufficiently in check, at least in so far as rotation can control them.

First of all, it may be said that even when peas are grown intensively, an adequate inspection of fields can always or nearly always detect the presence of disease before it has begun to reduce yields, so that the crop can be discontinued on infested soil before losses have occurred. New soils have never been found heavily infested with the fungus unless perchance there is a badly infested field near by. The disease first appears under usual conditions in small areas, whence it spreads in successive years to the rest of the field. Usually the disease does not reach serious extent until the second year after diseased plants can be readily found. Careful inspection of fields can be made an adequate safeguard against loss wherever there is economic advantage in growing peas intensively, provided peas are not returned to land on which disease has appeared for many years.

It is very difficult to predict how soon disease will begin to appear upon any given tract of land. Experience shows enormous variation in this regard. Sometimes the third successive crop is rendered unprofitable, while neighboring fields may grow peas for 5 or 6 years successfully, and there are instances known both in Wisconsin and in irrigated districts in which peas have been grown in fields nearly every year for 10 or 12 years without a single failure. There are two possible explanations for such differences in time before the appearance of disease; either the parasite may have been absent originally from the fields which grew peas without disease for the longer period, or some soil condition may have prevented its rapid distribution and development. There is some evidence which indicates that the first condition furnishes the explanation for the late appearance of disease in some districts where peas have not been grown previously. There is a widespread belief among growers that fields in a new district will grow peas successfully much longer than fields in a district where root rot is known. That this is not always true is shown by the following instance. In the summer of 1922 a canning factory in Wisconsin far north of any locality where peas have been grown intensively was growing its third crop of peas. One field had been planted the three years in succession, four had been planted two years, and the remaining fields were on new ground. When an examination of these fields was made, about 25 per cent of the plants in the field producing its third crop were found dying with root rot, a few small groups of infected plants were found in each of the fields producing the second successive crop; while no diseased plants were discovered in any of the several other fields that were carefully searched. Although it may be true that the absence or scarcity of the fungus from some localities may explain in part the tardy appearance of root rot, it is certainly difficult to obtain adequate evidence in support of such an opinion.

On the other hand, there is abundant evidence that the disease develops much more rapidly and severely in fields upon some soil types than in neighboring fields upon other types. For instance, fields on Superior red clay in Wisconsin appear to develop root rot earlier and more severely than on any other type of soil. A minor factor that may contribute to the severity of the disease upon this type of soil may be due to the fact that, because it can not usually be worked in the spring

as early as lighter types, planting is delayed, so that the plants are younger and more readily destroyed when conditions favorable for the disease develop. This condition does not furnish an adequate explanation for the prompt appearance of the disease with intensive culture on this soil, however. Another soil type upon which the disease develops with almost equal severity, though usually at a later stage in the development of the plant, is a very sandy soil in the trucking district south of Baltimore, Md. Although it is not obvious at first that there is any common condition in these extreme soil types that causes them to be favorable for the development of the disease, nevertheless observation of conditions in these districts and in others which will be cited later has led to the conclusion that it is the retention of water in both soils for a long period after rains that furnishes the favorable environment for the fungus. The Superior red clay is rather impervious to the passage of water and when once thoroughly saturated retains surface water in hollows and in the soil structure more persistently than do lighter types of soil. This offers conditions favorable for the germination of the oospores of Aphanomyces which may be present and perhaps for the production of zoospores. On the other hand, the sandy soil referred to is underlaid with an impervious subsoil, which, in more level fields, holds standing water in the lower portion of the sand for several days after heavy rains, thus providing a similar environment for the development of the parasite.

From this it appears that soil which is naturally retentive of water or in which water is held by reason of its relation to impervious layers furnishes the most favorable condition for the development of the disease. Such a supposition is rendered very plausible by the character of the fungus and is supported by other evidence, gathered not only in humid regions but in irrigated districts as well. The best examples of the relation of soil type and method of irrigation to the development of root rot have been found in the Gallatin and Paradise Valleys in Montana and near St. Anthony, Idaho. In the Gallatin Valley in 1921 this disease was found for the first time in a small field which appeared to have been overirrigated. The condition of the roots indicated that the infestation had developed after the plants were nearly grown, and although the root systems were thoroughly rotted the yield from this field was not apparently reduced. Since this was the first observed instance of the development of the disease in this valley, and it offered an excellent opportunity to obtain evidence which would indicate the severity which the disease might attain here, arrangement was made with the canning company for whom the peas were grown to have a portion of this field replanted with peas the following year. Such a field would not have produced an average crop of peas in a nonirrigated territory. Unfortunately the senior writer was unable to revisit this valley in the following year, but the canning company reported a normal yield of peas with no conspicuous evidence of disease. When the valley was revisited in 1923, a careful search of fields reported to have been used for this crop almost continuously for 10 or 12 years yielded but a single plant which was unmistakably diseased by this fungus. So far as this valley is concerned, there is no doubt that the fungus is present and that there are abundant facilities for its rapid distribution; yet, save possibly in cases of overirrigation, it appears that it is unable to become injurious. In contrast with conditions in this valley, fields in the Pine Creek district of the Paradise Valley about 25 miles distant from the Gallatin Valley were found thoroughly infested and greatly injured by root rot both in 1921 and 1923. Peas have been grown extensively in both valleys for at least 12 years under very similar climatic conditions. Whether a difference in soil structure or difference in the practice of irrigation has produced conditions favorable to the development of the disease in the Paradise Valley is not unmistakably apparent from observation.

No soil survey has yet been made of the Paradise Valley whereby soil formation there can be compared with that in the Gallatin Valley. There are, however, conspicuous differences in topography which suggest a difference in origin of the soils and a structure more retentive of water and conducive to seepage from higher levels in some fields of the Paradise Valley.

If the water-holding capacity of soil or the retention of water in the soil is so necessary for the development of root rot, we may ask again whether the apparent immunity from disease which some Wisconsin fields have shown may not be due to soil conditions which prevent the development of the parasite. Before a final answer is given to this question a large number of fields which

are known to have enjoyed this freedom from disease must be examined. In a conspicuous instance of such immunity examined recently, the subsoil of the field was found to be sand, which afforded admirable drainage. It may be that a limited amount of land will be found outside of irrigated territory on which peas may be grown intensively for a long time without trouble from root rot. However, most soil which has drainage and texture open enough to secure immunity to disease will probably be found too dry and infertile to produce large yields from a plant with a comparatively small root system like the pea.

Since there is a large amount of valuable land now incapable of producing profitable crops of peas because of infestation with root rot, the question is often asked how long the fungus remains in the soil, and if a rotation can be devised that will make possible the utilization of this land in the future. Since there is no experimental evidence which will aid in answering these questions, it is necessary to seek the experience of growers who have abandoned land for pea growing because of disease for varying periods of years. Experience of this kind is always open to question because one can not be sure that this root rot and not some other disease was the cause of the crop failure, both before and after the period in which the land was used for other crops. Notwithstanding such doubts, there appear to be two well-attested instances in Wisconsin in which fields failed to produce healthy peas after being used six years for other crops following a complete failure from rootrot. The writer has seen two fields of peas ruined by root rot after an interval of four years following a failure and another field in the same locality producing healthy peas nine years after a failure, presumably from this disease. In contrast with this Wisconsin experience, healthy fields of peas have been found in sandy soil in Delaware where peas failed four years earlier from disease which may have been root rot. From these instances and other experiences which might be cited it seems that in the heavier soils the parasite may persist for at least six years after it has once become abundant. In light soils its period of survival may be shorter. Probably no other fungus parasite of peas survives so long as this, and its duration in any locality can best be determined by repeated experimental plantings on infested ground during a series of years.

RESISTANT VARIETIES

Observation of the behavior of varieties of peas grown on diseased soil in several places has given rise to the opinion, more or less current among growers, that some varieties of peas show resistance and will produce a better crop than others. Inasmuch as Wilber Brotherton has been studying resistance under field conditions on an extensive scale and will report results in the near future, no attempt will be made here to consider the merits of commercial varieties. An attempt has been made to supplement Brotherton's studies by seeking to devise laboratory methods whereby disease resistance can be tested more rapidly than in field trials, and also to learn in so far as possible the nature of the resistance which varieties may possess.

The attempt to test varieties in the greenhouse under controlled conditions has not yet developed a method that is satisfactory. In the greenhouse in winter there is an apparent obliteration or reduction of differences in behavior compared with that observed in the field not unlike that which occurred in an earlier study (*8*, p. 471) of resistance of peas to a species of Fusarium. This work is still in progress and may be reported more fully later.

It may be said here, however, that no variety of peas, whether of the garden or of the field varieties, has been found immune to the fungus. The hardiest variety of field pea which produces a crop on soil so infested that few plants of garden varieties survive to maturity suffers almost if not quite as great a loss of cortex from its roots as its more susceptible neighbors. In spite of this loss it maintains growth and matures seed. This difference in behavior, termed resistance, seems to be due to several characters which resistant plants possess to a greater degree than those which perish. There is some evidence indicating that the fungus traverses the cortex of some varieties much more slowly than that of others, thus destroying roots less rapidly. Finally, when cortex is destroyed the remaining vascular cylinder of some plants seems to be able to exclude bacteria and fungi in the soil and to function quite efficiently thus denuded of absorbing tissue.

Whatever the importance of these several factors in resistance mentioned above, it will be seen at once that the growth of resistant peas

permits the infestation of the soil with the fungus to proceed as rapidly as when the most susceptible peas are grown. With intensive culture these resistant peas will be subjected to increasingly severe infection from year to year. Resistant varieties, however, may afford a very satisfactory escape from root rot where it is not extremely severe. But it remains to be determined experimentally whether any resistance has yet been found in varieties of commercial utility which will continue to produce profitable crops with intensive culture on soil in which this disease reaches its greatest severity.

SUMMARY

1. Of the several root-rot diseases of peas occurring in the United States which have been distinguished and studied during the past five years, the disease caused by the fungus described in this paper appears to be the most important. It occurs in nearly all of the important pea-growing districts with a varying severity which depends largely upon the degree to which intensive culture of peas permits the accumulation of the fungus in the soil and upon the conditions of soil temperature and moisture favoring early infection and rapid decay of the invaded roots. No other crop than peas has been found subject to disease from this fungus.

2. The effect of this disease upon the appearance of the plant in the field and upon the yield of the crop varies with the stage of development of the plant at which infection takes place and upon the number of infections. If the root system is invaded extensively when only three or four nodes have been formed, the plant may wilt and die suddenly. Later invasions may result in dwarfing of growth with drying out of foliage from the ground upward and in unproductivity. The disease can hardly be distinguished by the appearance of the top of the plant, but it can usually be identified readily when plants are pulled from the ground by the behavior of the root, which instead of breaking near the planted seed, pulls out as a fibrous string consisting of the vascular cylinder of the root freed from the decayed cortex.

3. The fungus enters only the cortex of the roots and base of the stem, where it produces a softening and rapid decay of the tissue, leaving the vascular cylinder exposed to decay by other organisms. In most varieties of garden peas the smaller roots thus denuded of cortex die immediately. A large number of oospores are formed by the fungus in the invaded cortex, and it appears to be from these spores, which increase in the soil from year to year with intensive culture of the crop, that infection originates each season.

4. The fungus can be isolated in pure culture only with considerable difficulty, both because the period during which the mycelium is growing actively in the tissue is brief and because it is so closely associated with bacteria and other fungi that the separation of the parasite from its associates is not readily accomplished. However, 12 cultures from different localities have been obtained for comparison and study.

5. Within the host tissues or on a suitable solid substratum the mycelium soon gives rise to resistant thick-walled bodies, the oospores which result from the development of oogonia following their fertilization by antheridia, of which from one to five are associated with each female cell. Depending on the presence or absence of food materials, the oospores germinate either directly by the production of one to several vegetative hyphae, or indirectly by the proliferation of a single germ hypha of limited growth, within which, as well as within the oospore wall, the protoplasm is divided into portions 13 to 18 in number, which are promptly discharged in the manner characteristic of the genus.

6. Asexual reproduction resulting in the formation of great numbers of motile zoospores ensues whenever actively growing mycelium is provided with suitable conditions. Young thalli may become almost entirely involved in sporogenesis, the individual sporangia being represented by portions of mycelium delimited by septa and often including a moderate number of well-developed branches. These sporangial units discharge their zoospores by one or several elements, the distal portions of which are considerably constricted.

7. The fungus shows much similarity to two aquatic congeners possessing smooth oogonia, *Aphanomyces laevis* and *A. helicoides*, differing especially, however, from the former in having a greatly thickened oogonial envelope with characteristically sinuous internal contour, and from the latter in its antheridial branches not exhibiting any well-defined spiral habit. It is described as a new species, *Aphanomyces euteiches* Drechsler.

8. Inoculation of pea plants with pure cultures under conditions of controlled soil temperature and moisture show that infection of peas may take place at temperatures between 10° and 30° C., but that optimum

temperature for development of the disease is approximately between 15° and 30°. Differences in soil moisture gave little difference in infection under the conditions of these experiments, whereas observation in the field seems to show that the disease is more severe on soil with high moisture-holding capacity or on soil in which water is held by impervious subsoil or by subirrigation.

9. The fungus appears to occur widely in cultivated soils and may be conveyed from infested fields to others by any agency that carries soil. There is no evidence indicating that it is carried with seed.

10. The disease can be prevented and controlled most effectively by crop rotation. The length of rotation required appears to vary greatly with local conditions. On certain irrigated soils which appear to have such low moisture-holding capacity that they provide unfavorable conditions for the development of the fungus, the disease has not appeared or has not become destructive even after peas have been grown nearly every year for 10 years. The disease has become destructive on similar soil when it is subirrigated. On some soils in humid territory the third successive crop of peas is often badly damaged by this root rot, and a comparatively long rotation appears to be necessary to prevent it from accumulating in the soil. In some soils which have become heavily infested the fungus appears to have persisted in sufficient amount to produce conspicuous injury to peas after a six-year rotation.

11. Although all varieties of field and garden peas are subject to this disease, there is considerable difference in the amount of injury which they incur from it, especially in situations where the disease does not develop great severity. Study of the nature and commercial value of this resistance is in progress.

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DIRECT INOCULATION OF CONIFEROUS STEMS WITH DAMPING-OFF FUNGI [1]

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INTRODUCTION

Hartley's (*2*, *3*) [2] and Spaulding's (*8*) summaries of the important literature on the damping-off of conifers have brought out the fact that aerial stem infections are less frequent than subterranean infections. Most of these published reports have been based upon field observations or upon experiments in which inoculum was added to the soil at the time the seeds were sown, instead of being applied directly to the seedlings. These methods, while giving the combined loss from the various types of damping-off, (germination loss, root damping-off, stem damping-off, etc.), are not entirely satisfactory for showing just what part of this loss each type causes. Results secured from the direct inoculation of coniferous taproots have been published previously (*6*). This paper will present the results from the direct inoculation of coniferous stems.

METHODS OF INOCULATION

The seedlings for most of the experiments were grown in autoclaved sand in 2-inch pots in the greenhouse of the United States Department of Agriculture at Washington, D. C. They were watered only with boiled water. The pots were kept from the time of seeding until the time of inoculation in glass-walled cupboards previously disinfected with mercuric chloride. At the time of inoculation the pots were taken from the cupboard and all but 10 seedlings were removed from each pot. The seedlings were inoculated while the seed-coats were still clinging to the cotyledons of most of them. The seedlings grown in this way, though normally green, were taller than seedlings of the same age usually are, the light intensity in the glass cupboards being apparently insufficient for normal growth.

The original plan was to inoculate each seedling at the soil surface because usually in seedbeds aerial infections occur at the soil surface rather than above. Almost immediately, however, this method was discarded in favor of inoculation platforms (fig. 1), since it was found very impractical to test the fungi by inoculation at the soil surface without the fungi's growing from one seedling to another through the soil. The method adopted is believed to give a fair indication of the results which would be obtained at the soil surface. The platforms consisted of equilateral triangles with sides three-eighths of an inch long cut from library cards. A toothpick was inserted through a hole in the center of each triangle. An inoculation platform was placed beside each seedling, and upon it in contact with the stem was placed the inoculum. Each toothpick was pushed into the soil until the triangular platform was about three-eighths of an inch above the soil surface.

Inoculum for each seedling consisted of either a 4 by 4 by 2 mm. cube of corn-meal agar, or rice mush about the size of a swollen rice grain, plus the mycelium and any spores growing upon it. In case both kinds of inoculum were used in the same experiments one kind was used on 5 seedlings, and the other kind on the 5 seedlings on the other side of the same pot. Each control pot was divided in the same manner, each half being treated with one kind of sterile medium. In each experiment there were usually 150 control seedlings, 75 for each medium when two kinds were used. At least 10 seedlings were inoculated with each fungous line grown on each medium.

Individual damp chambers were made for each pot, consisting of truncated sheet celluloid cones 5 inches high and 2½ inches in diameter at the base. Moisture was furnished by

[1] Received for publication, June 5, 1924—issued, April, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 339.

plugging the top of each cone with cotton wet in boiled water. In case the sand itself became too dry the pots were set in pans of boiled water. As soon as damp-chamber conditions were no longer desired (usually 2 days after inoculation) the cotton was removed from the top of the cone. Before use, the cones were disinfected with formalin or mercuric chloride solution.

After inoculation, the pots were placed on stands whose legs were set in pans of water in order to exclude slugs and ants. These methods of inoculation, together with others, have been described and illustrated in a previous paper (*4*). Each experiment was allowed to run four days. A daily count was made of the damped-off seedlings and those found diseased were removed. Only those seedlings which began to damp-off at the point of inoculation were considered (fig. 1).

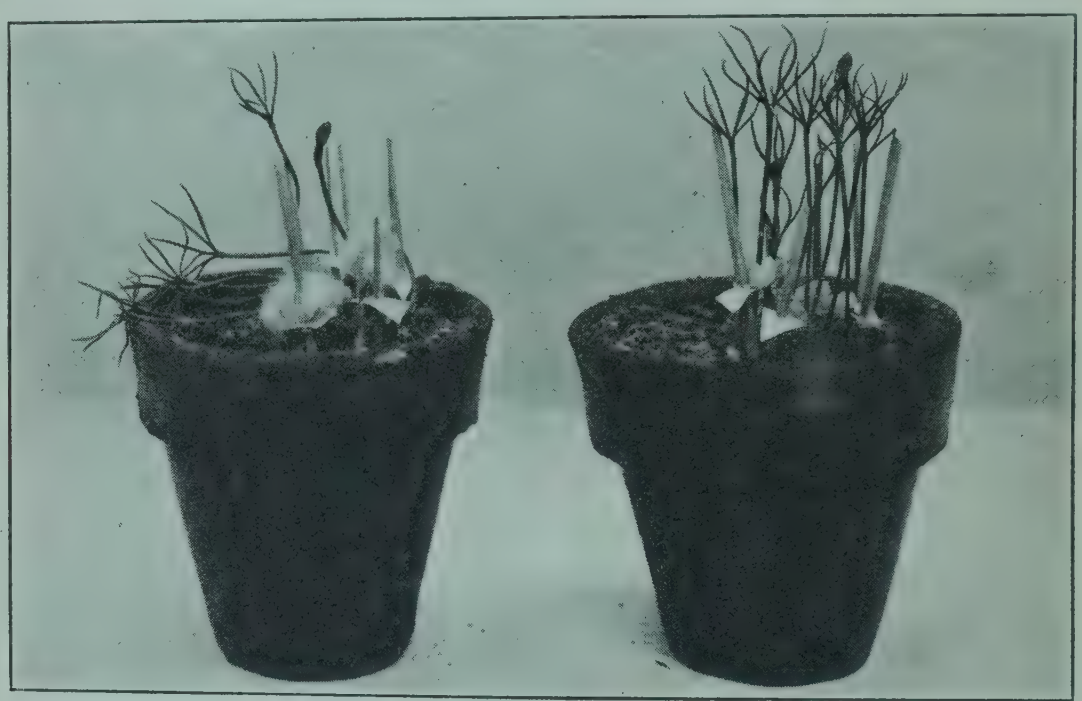

FIG. 1.—Pot at left shows damping-off caused by *Pythium debaryanum*. Pot at right shows seedlings treated at same time with sterile rice grains

It was undesirable to use the above method for such exotic fungi as *Phytophthora fagi* and *Pythiacystis citrophthora*. Therefore seedlings grown in plugged test tubes were inoculated with these two fungi. Sand was placed in plugged test tubes and the whole sterilized in the autoclave. Then the seeds were planted and watered with sterile water. In each tube were two or three seedlings. The inoculum which consisted of either corn meal or rice as above was placed on the surface of the sand in contact with the seedlings. The plug remained in the tube during the whole experiment, thus making a damp chamber. At the end of each experiment each tube was again autoclaved to prevent the escape of the fungi.

The results secured by the above methods of inoculation involving heavy inoculum for each seedling, seedlings grown on sterilized sand, boiled water, individual disinfected damp chambers, etc., do not constitute an entirely reliable index to the manner in which these fungi would act in ordinary seedbeds. They do, however, give some idea of the relative virulence and importance of the different genera of fungi and show which can attack coniferous stems under favorable conditions. The fungi which were only weakly parasitic in these tests would probably be entirely harmless under ordinary nursery conditions.

THE TESTED FUNGI

Over 100 different fungous lines were tested by the methods described above. By the term "line" as used in this paper is meant a fungous culture arising from one isolation. Although the cultures of Pythium, Corticium, and some others were isolated from tissue or soil plantings and were not entirely single lines, all except No. 230, maintained constant behavior through a sufficient number of generations to give fair assurance of their purity. Results obtained with many of the same lines have been published elsewhere (*2, 3, 5, 6*). In Table I are given

the line number, the host, the locality, and the person to whom each line originally belonged.

Technical Description of Phytophthora Sp.

A brief description is given below of the Phytophthora isolated from pine. This Phytophthora grew well on cornmeal agar, sterilized beans, peas, beets, and turnips.

Hyphae approximately 2.5 to 6.5 μ in diameter. Both aerial and submerged, flexuose and noduled with characteristic swellings at end of branches.

Sporangia approximately 19 to 40 μ by 28 to 86 μ. Not produced on ordinary media. Only produced when the hyphae alone, or the hyphae plus the substratum are placed in running water and kept at temperature of about 12° C. Germination by a tube or by formation of zoospores. Resting spherical zoospores approximately 7 to 9 μ.

Table I.—*Source and identity of the tested fungi* [a]

Name	Line No.	Source		
		Host	Locality	Contributed by—
Botrytis cinerea Pers.	470	*Pseudotsuga taxifolia* seedling.	Utah	C. Hartley.
	920	*Ceanothus americanus*	New York [b]	H. H. Whetzel.
	921	*Ribes rubrum*	do	Do.
	924	*Rosa* sp	do	Do.
	925	*Arachis hypogaea*	do	Do.
	944	*Hibiscus sabdariffa*	California	W. T. Horne.
Botrytis spp. (small sclerotial types).	922	*Arisaem atriphyllum*	New York [b]	H. H. Whetzel.
	923	*Hydrastis canadensis*	do	Do.
Cephalothecium roseum Cda.	288	*Ilex* nursery stock	District of Columbia	C. Hartley.
Corticium vagum B. and C. (*Rhizoctonia solani* Kühn)	50	*Pinus banksiana* seedling	Nebraska	Do.
	147	*Picea engelmanni* seedling	District of Columbia	Do.
	183	*Phaseolus vulgaris*	New York	M. F. Barrus.
	186	*Solanum tuberosum*	Ohio	Mrs. C. R. Tillotson
	187	do	New York	Do.
	189	*Beta vulgaris*	Michigan	Mrs. L. J. Weld.
	205	*Pseudotsuga taxifolia*	Colorado	Mrs. H. E. Watkins.
	213	*Beta vulgaris* seedling	District of Columbia	H. A. Edson.
	230	*Elaeagnus* sp	Garden City, Kans.	C. Hartley.
	233	*Elaeagnus sp.* (duplicate of No. 230).	do	Do.
	240	*Pinus ponderosa* seedling	do	T. C. Merrill.
	329	Reisolation of No. 147 from *Pinus ponderosa* seedling.		R. G. Pierce.
	330	Reisolation of No. 230 from *Pinus banksiana* seedling.		Do.
	331	Reisolation of No. 213 from *Pinus banksiana* seedling.		Do.
	332	Reisolation of No. 147 from *Pinus ponderosa*.		Do.
	333	Reisolation of No. 147 from *Pinus ponderosa* seedling.		Do.
	340	Reisolation of No. 213 from *Pinus banksiana* seedling.		Do.
	341	Reisolation of No. 230 from *Pinus banksiana* seedling.		Do.
	343	Reisolation of No. 147 from *Pinus banksiana* seedling.		Do.
	361	*Pinus resinosa* seedling	Cass Lake, Minn.	Do.
	362	do	do	Do.
	363	do	do	Do.
	365	do	do	Do.
	380	do	do	Do.
	381	*Pinus strobus* seedling	do	Do.
	552	*Picea engelmanni* seedling	California	C. Hartley.

[a] The writer wishes to express her thanks to Miss Helen Johann, of the Office of Cereal Investigations, U. S. Department of Agriculture, for the identification of Atanasoff's Fusarium cultures; to Dr. C. D. Sherbakoff, of the Tennessee Station, for the identification of several other Fusarium cultures; and to Dr. Carl Hartley, of the Office of Forest Pathology, U. S. Department of Agriculture, for his kind assistance and valuable suggestions.

[b] Locality not positive.

[c] Identified by C. D. Sherbakoff.

TABLE I.—*Source and identity of the tested fungi*—Continued

Name	Line No.	Source		
		Host	Locality	Contributed by—
Corticium vagum B. and C. (*Rhizoctonia solani* Kühn).	721	*Pinus resinosa* seedling	East Tawas, Mich.	G. G. Hahn.
	723	do	do	Do.
	724	do	do	Do.
	746	do	do	Do.
	747	*Pinus resinosa* seedling (duplicate of No. 746).	do	Do.
	761	*Pinus resinosa* seedling	do	Do.
Fusarium spp.	416	*Pinus banksiana* seedling	District of Columbia	R. G. Pierce.
	446	*Picea engelmanni* seedling	do	Do.
Fusarium acuminatum E. and E.	283	*Pinus ponderosa* seedling	do	T. C. Merrill.
	508	*Pseudotsuga taxifolia* seedling.	Utah	C. Hartley.
	932	*Ipomoea batatas*	District of Columbia	L. L. Harter.
Fusarium arthrosporioides Sherb.	908			D. Atanasoff.
Fusarium avenaceum (Fr.) Sacc.	911			Do.
Fusarium coeruleum (Lib.) Sacc.	934	*Solanum tuberosum*	New York[b]	H. A. Edson.
Fusarium culmorum (W. G. Sm.) Sacc. group.	[c]557	*Picea engelmanni* seedling	California	C. Hartley.
	917			D. Atanasoff.
	929	*Ipomoea batatas*	Indiana	L. L. Harter.
Fusarium discolor Ap. & Wr. section.	[c]367	*Pinus resinosa* seedling	Minnesota	R. G. Pierce.
	[c]518	Soil		C. Hartley.
	935		Germany	H. A. Edson.
Fusarium discolor sulphureum (Schlecht) Ap. & Wr.	936		do	Do.
Fusarium elegans group	[c]370	*Pinus resinosa* seedling	Minnesota	R. G. Pierce.
	[c]736	do	Michigan	G. G. Hahn.
	[c]779	do	do	Do.
	904			D. Atanasoff.
	909			Do.
	912			Do.
	913			Do.
	915			Do.
Fusarium eumartii Carp.	937	*Solanum tuberosum*	Pennsylvania	H. A. Edson.
Fusarium hyperoxysporum Wr.	930	*Ipomoea batatas*	District of Columbia	L. L. Harter.
Fusarium martiella section.	[c]268	*Pinus ponderosa* seedling	Kansas	T. C. Merrill.
Fusarium martii Ap. & Wr.	914			D. Atanasoff.
Fusarium moniliforme Sheld. section.	239	*Pinus ponderosa* seedling	Garden City, Kans.	T. C. Merrill.
	247	do	do	Do.
	249	do	do	Do.
	251	do	do	Do.
	260	do	do	Do.
	265	*Pinus banksiana* seedling	do	Do.
	266	*Pinus ponderosa* seedling	do	Do.
	273	do	do	Do.
	[c]308	Soil	do	R. G. Pierce.
	910			D. Atanasoff.
	[c]947	*Zea mays* roots	Louisiana	G. N. Hoffer.
	[c]948	*Zea mays* plant	Georgia	Do.
	[c]949	*Zea mays* stalk	Oklahoma	Do.
Fusarium orthoceras? Ap. & Wr.	[c]357	*Pinus resinosa* seedling	Minnesota	R. G. Pierce.
Fusarium oxysporum Schlecht.	938	*Solanum tuberosum*		H. A. Edson.
Fusarium radicicola Wr.	939	do	Colorado	Do.
Fusarium sp. between *roseum* and *discolor* groups.	[b]946	*Zea mays* stalks	Indiana	G. N. Hoffer.
Fusarium solani (Mart.) Ap. & Wr. group.	202	*Pinus ponderosa* seedling	Nebraska	C. Hartley.
	905			D. Atanasoff.
	940			H. A. Edson.
Fusarium sporotrichioides Sherb.	906			D. Atanasoff.
Fusarium trichothecioides Wr.	941	*Solanum tuberosum*	Idaho	H. A. Edson.
Fusarium vasinfectum Atk.	928	*Ipomoea batatas*	District of Columbia	L. L. Harter.
	942	*Gossypium*		H. A. Edson.
Fusarium ventricosum Ap. & Wr.	299	*Pinus ponderosa* seedling		C. Hartley.

[b] Locality not positive.

[c] Identified by C. D. Sherbakoff.

TABLE I.—*Source and identity of the tested fungi*—Continued

Name	Line No.	Source		
		Host	Locality	Contributed by—
Gibberella saubinetii (Mont.) Sacc.	916			D. Atanasoff.
Mucor racemosus Fres.	933	*Ipomoea batatas*	Indiana	L. L. Harter.
Nectria ipomoeae Hals.	927	do	do	Do.
Pestalozzia funerea? Desm.	931	*Ipomoea batatas*	New Jersey	Do.
	968	*Pinus palustris* leaves	Florida	M. W. Taylor and G. G. Hedgcock.
Phomopsis juniperovora Hahn.	860	*Juniperus virginiana*	East Tawas, Mich.	G. G. Hahn.
	864	do	do	Do.
	865	do	do	Do.
	867	do	do	Do.
Unidentified Phycomycete.	543	*Olea europaea*	California	C. Hartley.
Pythiacystis citrophthora Sm. and Sm.	943			J. T. Barrett.
Pythium artotrogus (Mont.) De Bary.	778	*Pinus resinosa* seedlings	Michigan	G. G. Hahn.
	821	*Pinus banksiana* seedlings	District of Columbia	Do.
	833	do	do	Do.
Pythium debaryanum Hesse.	131	*Solanum tuberosum*	California	Mrs. C. R. Tillotson.
	258	*Pinus ponderosa* seedling	Kansas	T. C. Merrill.
	295	Reisolation of No. 131 from *Beta vulgaris* seeding.		H. A. Edson.
	296	*Beta vulgaris* seedling	Wisconsin	Do.
	338	Reisolation of No. 295 from *Pinus ponderosa* seedling.		R. G. Pierce.
	408	Reisolation of No. 338 from *Pinus banksiana.*		Do.
	529	*Trigonella foenum graceum* seedling.	Sonoma County, Calif.	C. Hartley.
	550	*Picea sitchensis* seedling	Berkeley, Calif.	Do.
	743	*Pinus resinosa* seedling	Michigan	G. G. Hahn.
	767	do	do	Do.
	810	*Solanum tuberosum*	California	L. A. Hawkins.
Phytophthora cactorum (C. & L.) Sebrath.	901			L. O. Kunkel.
Phytophthora fagi Hart.	967			Joha. Westerdijk.
Phytophthora sp.	358	*Pinus resinosa* seedling	Cass Lake, Minn.	R. G. Pierce.
	372	do	do	Do.
	843	Reisolation of No. 372 from *Pinus ponderosa* seedling.		G. G. Hahn.
Rheosporangium aphanidermatus Edson.	351	*Beta vulgaris* seedling	Wisconsin	H. A. Edson.
	430	Reisolation of No. 351 from *Pinus banksiana* seedling.		R. G. Pierce.
Rhizoctonia potomacensis Wr.	881	*Lycopersicon esculentum*	District of Columbia	H. A. Edson.
Thielavia basicola (B. & B.) Zopf.	428	*Nicotinia*	Wisconsin	J. Johnson.
Verticillium sp.	[c]399	*Litchi chinensis*	California	R. G. Pierce.

[c] Identified by C. D. Sherbakoff.

Oogonia approximately 20 to 38 μ. Oospores approximately 14 to 31 μ. Oogonia brownish, smooth, practically spherical. Oospores smooth, spherical, lying free in oogonia. Walls about 2.6 μ thick. Oogonia apparently grow up through laterial or basal antheridia. Further taxonomic study is necessary.

METHOD OF EVALUATING RESULTS

The next to the last column in Tables II, IV, V, and VI shows the average per cent of the seedlings killed (direct average of the preceding three or four columns). It is obvious that this average does not absolutely represent the amount of damage which each fungus can cause, because it is based upon a direct comparison of the different fungi, just as if they had all been tested in the same experiment. As a matter of fact, some of the fungi were tested in one experiment, and others in different experiments in which conditions may have been more or less favorable to damping-off. Three "standard" fungi—one line of *Pythium debaryanum*, one of *Corticium vagum*, and one of *Fusarium moniliforme*—were used in all experiments for the sake of checking the homogeneity of the results. The fact that these fungi were decidedly more virulent in some

experiments than in others shows that the conditions of those experiments were more favorable than in others for damping-off. In order to make the results of the different experiments directly comparable the data were reworked. The mortality percentage for each fungus in each experiment was corrected, in much the same way that agronomists correct plot yields for field heterogeneity.[3] For example, if the average mortality per cent for the three standard lines in experiment B was less than the average of the same lines for all experiments, the mortality percentage for each fungus was proportionally raised. If the average mortality for them was more than it was for all experiments each mortality per cent was lowered. The corrected percentages were averaged and constitute the last columns in the tables. The averages in the next to the last column of the tables are open to suspicion because the percentages on which they were based are uncorrected for the varying conditions of the experiments. The corrected averages in the last column are under suspicion because of (1) the small number of standard lines on which corrections are based; (2) the probability that the respective fungi are differently affected by changes in conditions, and (3) the somewhat questionable character of the assumption on which the method of correction is based. It is thus probable that the corrected percentages are corrected too much and that the true mortality percentages lie somewhere between the corrected and uncorrected values. The fact that the agreement between the averages of the corrected and the uncorrected percentages is reasonably good, and that the relative virulence of the fungi seems approximately the same regardless of the method of averaging, seems to indicate that a direct comparison of the different experiments introduced no serious errors. An average of the uncorrected and corrected averages would probably be a fair index to the relative virulence of the fungi tested in these experiments.

TABLE II.—*Damping-off of coniferous stems caused by Pythium debaryanum and other Phycomycetes*

Tested fungi	Number of lines tested			Number of seedlings inoculated				Per cent of seedlings killed					Average of corrected percentages
	On Pinus resinosa	On Pinus banksiana	On Picea englmanni	Pinus resinosa		Pinus banksiana	Picea engelmanni	Pinus resinosa		Pinus banksiana	Picea engelmanni	Average	
				Agar inoculum	Rice inoculum			Agar inoculum	Rice inoculum				
Pythium debaryanum, all lines	11	9	9	330	460	160	230	57	90	98	97	85	83
Line No. 131 and its reisolations	4	3	3	80	120	50	80	62	91	100	96	87	83
Lines isolated from coniferous hosts	6	4	4	120	180	70	100	55	86	100	98	85	84
Lines isolated from other hosts	5	5	5	210	280	90	130	58	92	97	94	85	83
Rheosporangium aphanidermatus	2	2	----	40	40	40	-----	58	83	95	-----	79	72
Phytophthora sp	3	3	----	70	70	40	-----	14	34	25	-----	24	27
Phytophthora cactorum	1	----	----	20	20	-----	-----	0	15	-----	-----	8	13
Pythium artotrogus	3	1	----	60	60	20	-----	0	2	0	-----	1—	1
Unidentified Phycomycete	1	----	----	20	20	-----	-----	0	0	-----	-----	0	0
Mucor racemosus	1	----	----	10	10	-----	-----	0	0	-----	-----	0	0
Controls	----	----	----	975	1, 225	450	300	0. 1	2. 5	0. 7	0. 3	1—	------

[3] Obviously the percentages could not be corrected in exactly the same way however. For instance, if in all the experiments the standard lines caused an average loss of 60 per cent, but in experiment B only 40 per cent, a proportional correction of each mortality percentage in experiment B would raise a fungus which had caused 80 per cent damping-off to 120 per cent. Even in cases where there is no such obvious absurdity resulting from correction, the inevitable cramping of the percentage scale near 0 and 100 would necessitate some other method of applying corrections. The percentages on which the averages in the last columns of Tables II, IV, V, and VI were based, were corrected by the following method suggested by Dr. Sewall Wright.

It is evident that in experiment B of the above hypothetical case a mortality of 40 per cent caused by a particular fungus should be corrected to 60 per cent. In the normal frequency curve between the 40th and 60th percentile the abscissal distance on the base line is 0.5068σ. A 20 per cent loss caused by another fungus in the same experiment was corrected as follows:

By reference to a normal probability integral table (*1*, *Table IV*) it was found that an abscissal distance of 0.5068σ on the base line to the right of the 20th percentile had over it 17 per cent of the area of the normal probability curve. The 20 per cent mortality was therefore raised to 37 per cent. The same procedure raised 10 per cent to 20 per cent, 50 per cent to 69 per cent, and 90 per cent to 96 per cent. The basis of the method is the assumption that the conditions which affect a given lot of seedlings infected with a given fungus have a certain standard deviation which is the same for all lots.

RESULTS OF THE PRESENT INVESTIGATION

Pythium Debaryanum

The results secured by inoculating seedlings of *Pinus resinosa* Ait., *P. banksiana* Lamb., and *Picea engelmanni* Engelm. with *Pythium debaryanum* by the platform method are given in Table II. *P. debaryanum* caused some damping-off in all but three inoculated units, all three with the same weak line (No. 743). Regardless of its substratum, *P. debaryanum* caused more than half of the seedlings to damp-off. In the case of *Pinus resinosa* both rice mush and corn-meal agar inoculum were tested. In more than three-fourths of the tests the fungi when grown on rice mush caused more damping-off than in the same tests when grown on corn-meal agar. The latter were not more destructive in a single case. Since the mycelial development was much more luxuriant on the mush, it seems that virulence may be associated with vigor of growth. Figure 2 shows this graphically. The lines isolated from coniferous hosts were no more virulent than those isolated from other hosts. On the whole, the *P. debaryanum* lines were more destructive than any of the other fungi, with the possible exception of *Botrytis cinerea*. The control seedlings damped-off very little.

An attempt was made to get some idea of the relative susceptibility of the various hosts to *Pythium debaryanum* by comparing the percentages damped-off by the eight lines tested on all three hosts with rice mush as a substratum. The figures secured in this way vary slightly from those given in Table II, where all lines were included, but for practical purposes they are the same. For *Pinus resinosa* the damping-off was 93 per cent; for *P. banksiana*, 98 per cent; and for *Picea engelmanni*, 96 per cent. Thus it seems that all three host species are highly and practically equally susceptible to *P. debaryanum* under the conditions of these experiments. A comparison of results secured with the standard Pythium line confirms this conclusion.

Some of the *Pythium debaryanum* lines were used in miscellaneous small experiments with other coniferous seedlings. This fungus caused 26 out of 30 seedlings of *Abies nobilis* Lind. to damp-off in an experiment in which 1 out of 10 control seedlings damped-off. Because of the difficulty of germinating the Abies seeds it was impossible to conduct other or larger experiments. For the same reason only small experiments were conducted with *Pinus caribaea* Morel. There was no damping-off among the 20 control seedlings, although 18 out of the 30 inoculated ones damped-off. One out of 10 control seedlings of *Pseudotsuga taxifolia* (Lam.) Brit. and 15 out of 20 inoculated ones damped-off. The above tests are purely preliminary but seem to indicate that *Pythium debaryanum* can cause damping-off of *A. nobilis*, *Pinus caribaea*, and *Pseudotsuga taxifolia*. The first two are new hosts for *P. debaryanum*, and heretofore it had not been definitely proven that the last named is a host for it.

Two small test tube experiments and two pot experiments in which seedlings of *Pinus resinosa* were inoculated at the ground level with three of these *Pythium debaryanum* lines confirmed the results secured with the same lines by the platform method.

Other Phycomycetes

The results secured by inoculating seedlings of *Pinus resinosa* and *P. banksiana* with miscellaneous Phycomycetes by the platform method are also given in Table II.

RHEOSPORANGIUM APHANIDERMATUS

The two available lines of *Rheosporangium aphanidermatus* were very parasitic to both species, being approximately as virulent as *Pythium debaryanum* itself, and decidedly more virulent than most of the other fungi. On the whole it was more active when grown on rice mush than when grown on corn meal agar (fig. 2).

MUCOR RACEMOSUS

This species was tested on only 20 seedlings in an experiment in which the standard fungi were less virulent than the average, and caused no disease at all. An unknown Phycomycete, which formed only small irregular chlamydospores, caused no disease and the spiny Pythium, which has been referred to the species *artotrogus* caused no appreciable damping-off. In Hartley's (*3*) experiments *P. artotrogus* caused somewhat more damping-off than in the writer's experiments.

PYTHIACYSTIS CITROPHTHORA

To avoid the danger of establishing this exotic form in the eastern United States, it was used in a small test-tube

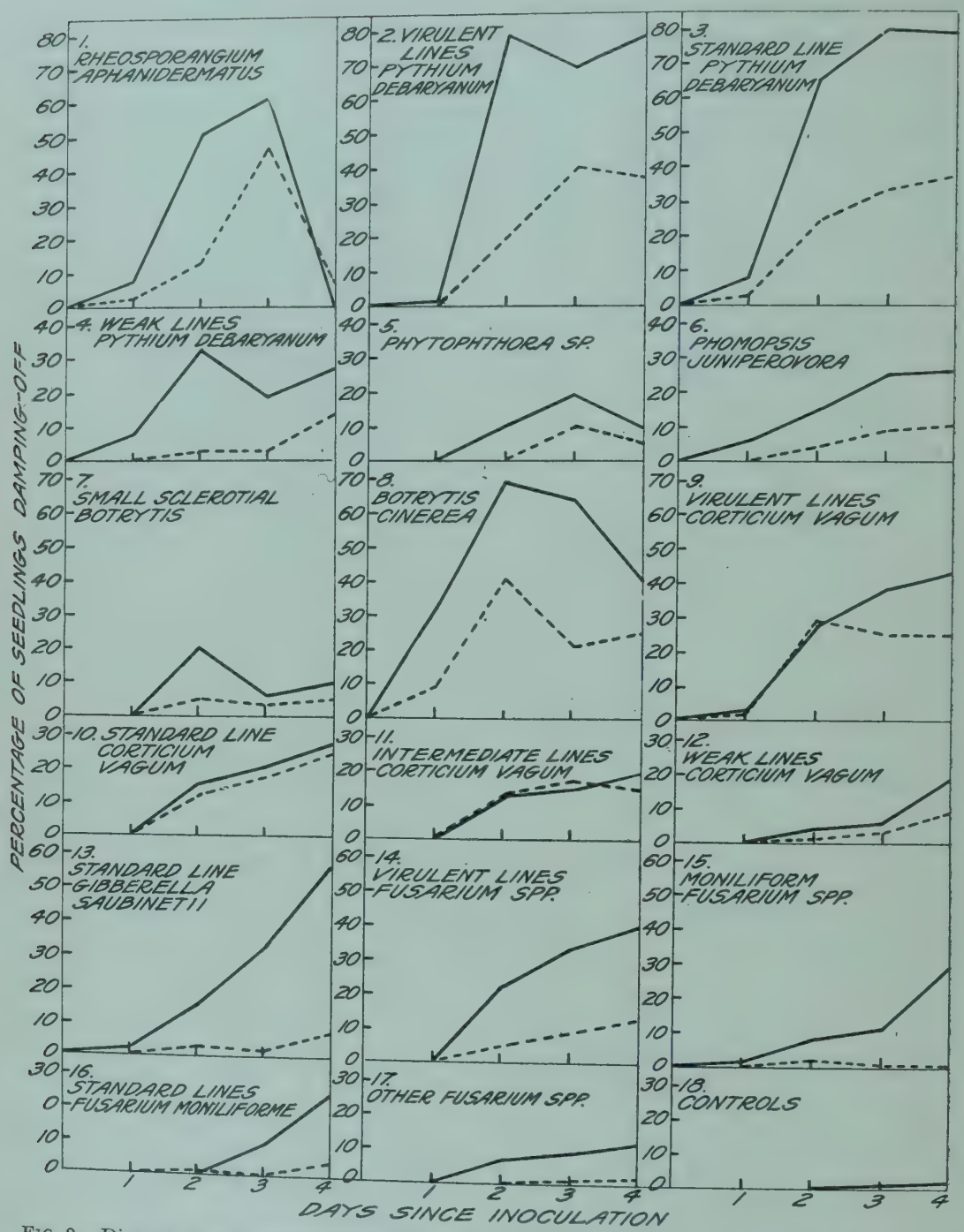
1. RHEOSPORANGIUM APHANIDERMATUS
2. VIRULENT LINES PYTHIUM DEBARYANUM
3. STANDARD LINE PYTHIUM DEBARYANUM
4. WEAK LINES PYTHIUM DEBARYANUM
5. PHYTOPHTHORA SP.
6. PHOMOPSIS JUNIPEROVORA
7. SMALL SCLEROTIAL BOTRYTIS
8. BOTRYTIS CINEREA
9. VIRULENT LINES CORTICIUM VAGUM
10. STANDARD LINE CORTICIUM VAGUM
11. INTERMEDIATE LINES CORTICIUM VAGUM
12. WEAK LINES CORTICIUM VAGUM
13. STANDARD LINE GIBBERELLA SAUBINETII
14. VIRULENT LINES FUSARIUM SPP.
15. MONILIFORM FUSARIUM SPP.
16. STANDARD LINES FUSARIUM MONILIFORME
17. OTHER FUSARIUM SPP.
18. CONTROLS
PERCENTAGE OF SEEDLINGS DAMPING-OFF
DAYS SINCE INOCULATION

FIG. 2.—Diagram showing time elapsing between inoculation and the appearance of damping-off in *Pinus resinosa* seedlings. The percentage of seedlings damped-off each day is based upon the number of seedlings living at the end of the preceding day. The solid line ——— indicates that rice mush was used as the substratum for the inoculum; the broken line ------ indicates that corn-meal agar inoculum was used. Only those experiments are reported in which both kinds of inoculum were tested simultaneously. The standard fungi whose effect is shown in graphs 3, 10, 13, and 16 are those lines used in all tests for the sake of comparison; the results with these are not included in the other graphs. The following graphs represent results with more than one line: No. 1, 2 lines; No. 2, 7; No. 4, 3; No. 5, 3; No. 6, 4; No. 7, 2; No. 8, 6; No. 9, 10; No. 11, 8; No. 12, 8; No. 14, 5; No. 15, 11; No. 16, 2; No. 17, 26. Each of the ordinary lines, with some unimportant exceptions, was tested on 20 seedlings with each kind of inoculum and the standard lines (except Gibberella) on 130 or more seedlings on each inoculum. The controls included approximately 1,000 seedlings on each inoculum

experiment with *Pinus resinosa* and caused 100 per cent damping-off compared with 7 per cent in the control plants. This confirms an earlier greenhouse experiment in which inoculations with this fungus at the surface of the soil killed all the seedlings. This is a new host for Pythiacystis.

PHYTOPHTHORA FAGI

Seedlings of *Pinus resinosa* were inoculated with *Phytophthora fagi* in three small test-tube experiments. The results are given in Table III.

TABLE III.—*Damping-off of seedlings of Pinus resinosa caused by Pythium debaryanum and Phytophthora fagi*

	First experiment, per cent of damping-off	Second experiment, per cent of damping-off	Third experiment, per cent of damping-off
Controls	7	6	6
Pythium debaryanum	80	100	100
Phytophthora fagi	50	47	67

PHYTOPHTHORA CACTORUM

This species which Rosenbaum (7) combines with *P. fagi* caused practically no damping-off, but was used only in experiments in which, as is shown by results with the standard fungi, conditions were less favorable than usual for damping-off. Like *P. fagi*, it was obviously less virulent than *Pythium debaryanum*. Further comparative results with it are greatly to be desired.

PHYTOPHTHORA SPECIES

Three lines of a Phytophthora species isolated from pine caused 23 per cent, 43 per cent, and 54 per cent damping-off in the above test-tube experiment in which *P. fagi* caused 67 per cent. In a single experiment one line caused 100 per cent of the seedlings to damp-off. All three lines caused some damping-off of both *Pinus resinosa* and *P. banksiana* with the platform method of inoculation (Table II).

Obviously *Phytophthora cactorum*, *P. fagi* and the pine Phytophthora are less virulent than *Pythium debaryanum*. They are kept separate in this paper because of the need of more extensive taxonomic study and because of the difference in results.

CORTICIUM VAGUM (RHIZOCTONIA SOLANI KÜHN)

The results of inoculating *Pinus resinosa*, *P. banksiana* and *Picea engelmanni* with *Corticium vagum* lines are given in Table IV. The Corticium lines exhibited considerable variability, and tests of the same lines at different times did not agree very well. One possible explanation of this may be the fact that most of the lines were used in two large experiments, one when it was very hot and the other when it was very cold. This suggests the theory that the virulence of *Corticium vagum* may be closely associated with temperature. Taken as a whole, the *Corticium vagum* lines did not kill half of the inoculated seedlings. However, the most virulent third killed three-quarters of them. In other words, they were more virulent than the standard lines of Corticium or Fusarium, but less virulent than the standard Pythium line. The lines isolated from coniferous hosts were apparently no more virulent from those isolated from other hosts.

The difference between the results secured from the use of corn-meal agar and rice mush as substrata for *Corticium vagum* was comparatively little. In slightly more than half of the units the lines growing on agar caused as much or more damping-off than the same lines growing on rice. Corn-meal agar seemed to be a better substratum for Corticium than for any other fungus tested (fig. 2). A comparison of the lines exclusive of the standard one (No. 147) used on all three hosts gives the following results: For *Pinus resinosa*, 55 per cent damping-off; for *P. banksiana*, 49 per cent, and for *Picea engelmanni*, 24 per cent. A comparison of all the lines used on *P. banksiana* with the same ones on *P. resinosa* gave, respectively, 50 per cent and 49 per cent damping-off. From these results one would assume that the two pine species are of approximately equal susceptibility, while the spruce is less susceptible. However, further experiments are necessary to establish relative susceptibility.

Two preliminary tests in which the seedlings were inoculated with several Corticium lines at the soil surface in general confirmed the results secured by the platform method.

Two lines of *Corticium vagum* killed 7 out of 20 seedlings of *Pinus caribaea* in an experiment in which none of the 20 control seedlings damped-off. This is a new host for Corticium.

TABLE IV.—*Damping-off of coniferous stems caused by Corticium vagum*

Tested fungi	Number of lines tested			Number of seedlings inoculated				Per cent of seedlings killed					
				Pinus resinosa				Pinus resinosa					
	On Pinus resinosa	On Pinus banksiana	On Picea engelmanni	Agar inoculum	Rice inoculum	Pinus banksiana	Picea engelmanni	Agar inoculum	Rice inoculum	Pinus banksiana	Picea engelmanni	Average	Average of corrected percentages
Corticium vagum:													
All lines	30	23	12	680	730	250	140	36	46	45	36	41	39
Lines isolated from coniferous hosts	23	17	10	550	580	190	130	37	44	39	36	39	37
Lines isolated from other hosts	7	6	2	130	150	60	10	30	51	63	0	38	41
Line No. 147	1	1	1	130	160	30	30	44	58	37	77	54	61
Reisolations of line No. 147	4	3	2	70	70	30	20	71	50	40	5	42	35
Line No. 213	1	1	----	20	20	10	-----	70	75	100	-----	82	85
Reisolations of line No. 213	2	2	1	40	40	20	10	55	68	55	60	60	62
Line No. 230	1	1	----	20	20	10	-----	50	100	100	-----	83	81
Reisolations of line No. 230	3	3	1	60	60	30	10	40	50	33	30	39	43
Line No. 240	1	1	1	20	20	10	10	75	100	100	90	91	93
Rhizoctonia potomacensis [a]	1	1	1	20	20	10	10	5	35	30	0	18	18
Controls	----	----	----	975	1, 225	450	300	0. 1	2. 5	0. 7	0. 3	1—	------

[a] Only a line of *Corticium vagum*, according to H. A. Edson, who furnished it.

TABLE V.—*Damping-off of coniferous stems caused by Fusarium spp.*

Tested fungi	Number of lines tested			Number of seedlings inoculated				Per cent of seedlings killed					
				Pinus resinosa				Pinus resinosa					
	On Pinus resinosa	On Pinus banksiana	On Picea engelmanni	Agar inoculum	Rice inoculum	Pinus banksiana	Picea engelmanni	Agar inoculum	Rice inoculum	Pinus banksiana	Picea engelmanni	Average	Average of corrected percentages
Fusarium sporotrichioides	1	1	1	20	20	10	10	25	100	90	80	74	83
Fusarium discolor sulphureum	1	----	----	20	20	-----	-----	50	90	-----	-----	70	54
Fusarium arthrosporioides	1	1	----	20	20	10	-----	15	65	40	-----	40	52
Fusarium trichothecioides	1	----	----	20	20	-----	-----	40	35	-----	-----	38	20
Gibberella saubinetii	2	1	1	80	90	30	30	8	56	50	27	35	27
Fusarium hyperoxysporum	1	1	----	20	20	10	-----	0	55	50	-----	35	32
Fusarium sp. between roseum and discolor groups	1	1	1	20	20	10	10	0	55	20	40	29	28
Fusarium moniliforme species and section:													
All lines	13	11	6	380	410	130	80	5	41	28	26	25	19
Lines isolated from coniferous hosts	8	7	3	280	310	90	50	7	41	34	26	27	20
Lines isolated from other hosts	5	4	3	100	100	40	30	0	40	18	7	16	15
Nectria ipomoeae	1	----	----	20	20	-----	-----	10	40	-----	-----	25	18
Fusarium martiella section	1	1	----	20	20	10	-----	0	5	70	-----	25	24
Unidentified Fusarium spp	2	----	----	40	40	-----	-----	3	40	-----	-----	22	18
Fusarium solani group	3	1	----	60	60	10	-----	0	17	50	-----	22	19
Fusarium orthoceras?	1	----	----	20	20	-----	-----	10	30	-----	-----	20	40
Fusarium martii	1	1	----	20	20	10	-----	5	15	20	-----	13	18
Fusarium culmorum group	3	3	1	60	60	30	10	3	45	0	0	12	12
Fusarium radicicola	1	----	----	20	20	-----	-----	10	10	-----	-----	10	4
Fusarium elegans group	8	2	1	150	170	20	10	1	19	15	0	9	10
Fusarium avenaceum	1	----	----	20	20	-----	-----	0	15	-----	-----	8	12
Fusarium coeruleum	1	----	----	20	30	-----	-----	0	13	-----	-----	7	2
Fusarium acuminatum	3	1	----	60	60	10	-----	2	7	10	-----	6	4
Fusarium vasinfectum	2	1	----	40	40	10	-----	3	13	0	-----	5	7
Fusarium discolor section	3	1	----	40	60	10	-----	0	5	10	-----	5	5
Fusarium ventricosum	1	----	----	20	20	-----	-----	0	0	-----	-----	0	0
Fusarium oxysporum	1	1	----	20	20	10	-----	0	0	0	-----	0	0
Fusarium eumartii	1	----	----	20	20	-----	-----	0	0	-----	-----	0	0
Controls	----	----	----	975	1, 225	450	300	0. 1	2. 5	0. 7	0. 3	1	------

Fusarium spp.

The results of inoculation with various species of Fusarium are given in Table V. *F. sporotrichioides* and *F. discolor sulphureum* were very parasitic, comparing favorably in virulence with the more parasitic Pythium and Corticium lines. The species of the moniliform section of Fusarium ranged in virulence from the very parasitic to the almost nonparasitic forms. *F. arthrosporioides*, *F. hyperoxysporum*, *F. trichothecioides*, and *Gibberella saubinetii* were all moderately parasitic. *F. eumartii*, *F. oxysporum*, and *F. ventricosum* were definitely nonparasitic under the conditions of these experiments. *F. acuminatum*, *F. avenaceum*. *F. coeruleum*, certain lines of the *F. discolor* groups, *F. martii*, *F. radicicola* and *F. vasinfectum* were either nonparasitic or at times very doubtfully parasitic. It is not probable that any of these would cause much trouble under nursery conditions. Certain lines of the *F. culmorum* group, *F. martiella*, *Nectria ipomoeae*, *F. orthoceras*, *F. solani* and some unidentified *Fusarium* spp. showed evidence at times of being intermediate between the very parasitic and the nonparasitic groups. Further experiments are absolutely necessary before the ability of either of these last two classes to attack conifers is established.

In no case where there was a definite measurable degree of parasitism was Fusarium so active on corn-meal agar inoculum as on rice mush. (Table V and fig. 2.) In one small experiment with *Pinus caribaea* in which none of the 10 control seedlings died and in which 4 of the 10 seedlings inoculated with *Pythium debaryanum* damped-off, *Fusarium moniliforme* caused 3 out of 10 seedlings to damp-off. This purely preliminary experiment indicates *P. caribaea* as a new host for *F. moniliforme*.

Botrytis spp.

Table VI gives the results of inoculating *Pinus resinosa* and *P. banksiana* with *Botrytis* spp. The two available small sclerotial forms of Botrytis were either very slightly parasitic or entirely nonparasitic on both pine hosts. When grown on rice mush one of them seemed fairly parasitic to *P. resinosa*. It is probable, however, that this specialized parasite under ordinary conditions would be of no importance on coniferous seedlings.

The large sclerotial forms of Botrytis were all much alike and were all of the type which has previously gone under the name of *Botrytis cinerea*. It is considered best to continue the use of that generally known name until there is published warrant for change or segregation. These large sclerotial forms were more parasitic than the standard Fusarium line. They were always as destructive, and in most cases more so, than the standard Corticium line, and in all but one experiment, fully equaled the standard *Pythium debaryanum*. It is a less important parasite for coniferous seedlings than the latter, because in only a few cases has it been isolated from damped-off coniferous seedlings.

A comparison of the damping-off caused by the same lines of *Botrytis cinerea* on *Pinus resinosa* and *P. banksiana*, gave in both cases a result of 94 per cent, both species appearing highly and equally susceptible to them. There is a slight difference between

Table VI.—*Damping-off of coniferous stems caused by miscellaneous fungi*

Tested fungi	Number of lines tested		Number of seedlings inoculated			Per cent of seedlings killed				
			Pinus resinosa			Pinus resinosa				
	On Pinus resinosa	On Pinus banksiana	Agar inoculum	Rice inoculum	Pinus banksiana	Agar inoculum	Rice inoculum	Pinus banksiana	Average	Average of corrected percentages.
Botrytis cinerea:										
All lines	6	4	130	130	70	65	95	94	86	86
Lines isolated from coniferous hosts	1	1	20	20	20	50	90	80	73	68
Lines isolated from other hosts	5	3	110	110	50	72	96	100	89	92
Phomopsis juniperovora	4	4	70	90	80	21	53	66	47	35
Cephalothecium roseum	1	1	20	20	20	30	45	35	37	14
Small sclerotial Botrytis	2	2	40	40	40	13	32	5	17	12
Pestalozzia funerea?	1	1	20	20	20	0	20	0	7	7
Verticillium sp	1	1	20	20	20	0	0	0	0	0
Thielavia basicola	1	1	10	10	20	0	0	0	0	0
Controls	------	------	975	1,225	450	0.1	2.5	0.7	1	------

the above figure and the ones given in Table VI because more lines were used on *P. resinosa* than on *P. banksiana*. Isolation from a coniferous host did not increase virulence of *Botrytis cinerea*.

In only 6 per cent of the tested cases was Botrytis more virulent when grown on corn-meal agar than when grown on rice mush. The virulence was, however, equal in 41 per cent of the cases. The different lines exhibited more variability when grown on agar than when grown on mush (fig. 2). Four lines showed the same virulence when the inoculum was placed at the ground level as when it was placed on platforms.

Miscellaneous Fungi

Table VI gives the results of inoculating *Pinus resinosa* and *P. banksiana* with miscellaneous fungi by the platform method.

All lines of *Phomopsis juniperovora* showed some evidence of parasitic ability. There is some slight evidence that *Pinus banksiana* is slightly more susceptible than *P. resinosa* to this species. Hartley (*2*) and his associates previously found the species unable to cause damping-off in soil inoculations. There is a possibility that the reason for this is the inability of the fungus to maintain itself in the soil saprophytically until the seed has sprouted.

Cephalothecium roseum showed some slight evidence, which needs confirmation, of ability to cause damping-off of both species. *Thielavia basicola, Pestalozzia funerea*? and an unidentified *Verticillium* sp. caused no damping-off.

TIME ELAPSING BETWEEN INOCULATION AND APPEARANCE OF DAMPING-OFF

As stated above, all experiments were continued for four days. Figure 2 gives a set of graphs comparing the daily damping-off of *Pinus resinosa* seedlings when inoculated with various groups of fungi grown on rice mush and corn-meal agar. The percentages for each day are based upon the number of seedlings living at the end of the preceding day.

Pythium debaryanum, Rheosporangium aphanidermatus, Phomopsis juniperovora and the more virulent *Corticium vagum* lines caused the collapse of some seedlings within 24 hours from the placing of the inoculum, while *Botrytis cinerea* caused almost one-third of the seedlings to damp-off during the same period. With the exception of Phomopsis, these same fungi caused about the same percentage of *P. banksiana* seedlings to damp-off by the end of the first day. There was a slight amount of damping-off caused by *Fusarium* spp. during the same period. By the end of the first day *P. debaryanum* caused a somewhat larger percentage of the *Picea engelmanni* seedlings to damp-off.

At the end of the fourth day for most of the Fusarium and Corticium groups the percentage of damping-off was increasing (fig. 2). This probably means that the experiments were closed before some of these lines had showed their maximum parasitic ability. This fact may explain in part why in these experiments Corticium seemed less virulent than formerly (*3*). Most of the other fungi seemed to have reached their maximum parasitic ability by the end of three days.

Botrytis cinerea, Rheosporangium aphanidermatus, and *Pythium debaryanum* all grow very rapidly on artificial media. This may explain in part why damping-off symptoms appeared so soon on seedlings inoculated with them. However, Phomopsis grows slowly. The first three genera are also all very virulent parasites. It is rather surprising in view of their reputation for toxicity that the more parasitic of the *Fusarium* spp. were so much slower than Pythium in producing a visible effect on the seedlings.

Ten Fusarium lines and a few Corticium lines which always gave negative results were omitted from the graphs. No damping-off was noticeable in the controls until the end of three days.

DISCUSSION OF RESULTS

Lines of *Pythium debaryanum, Rheosporangium aphanidermatus, Corticium vagum, Botrytis cinerea*, and a few of the numerous species of Fusarium were all able to cause considerable damping-off. *Pythium debaryanum*, the very similar Rheosporangium, and *Botrytis cinerea* seem able to damp-off approximately the same percentage of the inoculated seedlings. However, in view of the fact that Hartley and his assistants (*2*) found Botrytis causing damping-off only in the laboratory and never in the nursery bed, and have never found Rheosporangium occurring naturally in coniferous seedlings, less importance is attached to inoculation results with them. They are very active parasites and are potentially capable of causing great damage, if present under nursery conditions.

Under the conditions of the writer's experiments, the *Pythium debaryanum* lines showed a smaller virulence range than did the same ones when used in soil inoculation experiments by Hartley (*2*). There are two possible explanations for this: (1) Some lines may have formerly been considered nonparasitic because of their inability to live saprophytically in the soil until the seeds had germinated, and (2) lines really weak may have seemed strongly parasitic in the writer's tests because of the heavy inoculum. The same may be true also of other genera.

For unexplained reasons, *Corticium vagum* exhibited far more variability than it did in Hartley's (*2*, *3*) experiments.

Few of the *Fusarium* species exhibited decidedly high or constant parasitic ability, but as a whole the genus appears able to cause considerable damage.

The other tested fungi besides *Pythium debaryanum, Corticium vagum, Botrytis cinerea* and *Fusarium* spp. are probably unimportant in the field because of the infrequency of their occurrence in coniferous nurseries even though some lines tested in these experiments were rather virulent.

The fact that the virulence and variability of the parasites depend to a certain extent on the medium used as inoculum, suggests that the virulence of the parasites in the soil, or at least their ability to maintain themselves saprophytically there until seeds had germinated, is largely dependent upon the amount and quality of the available food supply.

In order definitely to establish the relative susceptibility of the various hosts, tests should be conducted in which all the hosts are inoculated in the same experiment. However, it is to be expected that the hosts would be of approximately equal virulence in these experiments because Hartley (*3*) reported *Pinus resinosa, P. banksiana,* and *Picea engelmanni* as highly susceptible to damping-off under field conditions. It is probable that the fungi which proved themselves only moderately parasitic under the extremely artificial conditions of these experiments would do little or no damage under nursery conditions.

SUMMARY

(1) Direct inoculations of stems without bringing the inoculum in contact with the soil were conducted in the greenhouse of the United States Department of Agriculture at Washington, D. C. with seedlings of *Pinus resinosa, P. banksiana,* and *Picea engelmanni.* Over one hundred different fungous lines, most of which were *Pythium debaryanum, Corticium vagum,* and *Fusarium* spp., were tested.

(2) The virulence and variability of the different fungi depend to some extent on the substratum on which they are grown.

(3) The most virulent parasites in these experiments were *Pythium debaryanum. Botrytis cinerea, Rheosporangium aphanidermatus* and *Fusarium sporotrichioides.*

(4) The time elapsing between inoculation and the appearance of damping-off was especially short for the virulent swift-growing fungi.

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ROOT ROT OF THE GRAPEVINE IN MISSOURI CAUSED BY CLITOCYBE TABESCENS (SCOP.) BRES.[1, 2]

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INTRODUCTION

Shortly after the writer had assumed his duties at the Missouri State Fruit Experiment Station in 1922 his attention was directed to reports received from several localities of the destruction of grapevines caused by root rot. The first opportunity to investigate this disease was afforded by a trip to Knobview, Mo., on May 30, 1922, where, in the vineyard from which the trouble was reported, a number of vines that had recently died were carefully dug up. All exhibited the same symptoms, namely, an abundant growth of characteristic whitish to isabelline mycelial sheets or mats occurring between the layers of the innner bark and between the bark and the wood of the roots and root crown, occasionally accompanied by black rhizomorphic strands. These symptoms indicated that the disease was caused by a mushroom root rot, although no fruiting bodies could be found to verify the supposition. Cultures and further field studies were made which afforded a basis for the conclusions herein reported.[3]

HISTORICAL REVIEW

Although grapevines are known to be attacked by several different root rotting fungi, especially when the root systems have become weakened or injured, comparatively few investigations have been made in this country of the resulting diseases.

Root rot of the grapevine has long been known in Europe, where it has been investigated extensively. In France it is called "blanc des racines" or, more commonly, "pourridié;" in Germany "Weinstock-fäule;" and in Australia and some other countries "white-rot." According to Verge (*56*)[4] the cause of pourridié in Europe is attributed to several of the higher fungi, among them being *Armillaria mellea* (Fries) Quél., *Dematophora necatrix* Hartig, *Vibrissea* (*Roesleria*) *hypogaea* Ch. Richon and Le Monnier, and sometimes *Psathyrella ampelina* Foex and Viala. In the United States the disease has sometimes been attributed to these fungi and also to *Phymatotrichum* (*Ozonium*) *omnivorum* (Shear) Duggar. Most American writers, however, have generally believed that the disease in this country is caused by *Armillaria mellea* and *Dematophora necatrix* (*51, p. 172–174*), although as a rule on assumption rather than from definite evidence.

Root rot of the grapevine was first reported in this country in Missouri, Scribner (*46, p. 137*) stating that it was discovered in a vineyard at Bushberg, about 25 miles south of St. Louis, by the eminent French viticulturist Viala, in 1887, while the two were making a tour of the principal grape-growing regions of the United States. The same author also states (*47, p. 12; 48, p. 64*) that they found the disease later in northeastern Texas and again in Napa Valley, Calif. He records the fact that he has seen a number of vines nearly or quite dead from the same disease in the vicinity of Knoxville, Tenn.

In 1892 Pierce (*38 p., 153–161*), discussing the relation of root-attacking fungi to the California viné disease, states that Dematophora, although found in several places in the United States, had not been seen in California; that *Armillaria mellea* has been reported in northern California; and that *Vibrissea hypogaea* was found in

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[1] Received for publication May 19, 1924; issued April, 1925.

[2] Commonly known in this country under the names *Clitocybe monadelpha* (Morg.) Sacc., *Armillaria mellea exannulata* Peck, *Clitocybe parasitica* Wilcox, and *Monadelphus caespitosus* (Berk.) Murrill.

[3] The writer gratefully acknowledges helpful advice and suggestions concerning the taxonomic treatment of the fungus under discussion from Dr. E. A. Burt of the Missouri Botanical Garden, Dr. L. O. Overholts of the Pennsylvania State College, and C. G. Lloyd of the Lloyd Library and Museum at Cincinnati. Grateful acknowledgment is made of the invaluable services rendered by the libraries of the Missouri Botanical Garden and the U. S. Department of Agriculture.

[4] Reference is made by number (italic) to "Literature cited," p. 364.

the Santa Ana Valley on the roots of vines which had been brought from Missouri. He also reports the occurrence of a species of Graphium infesting diseased vine roots in the Santa Ana Valley but thinks that it bore no relation to the cause of the disease in question.

Between this date and 1900 a few additional reports were made of grapevine root rot. McCarthy (*25, p. 122*), discussing the disease in North Carolina, and Starnes (*50, p. 282*), in Georgia, both arbitrarily attribute it to *Dematophora necatrix* and *Armillaria* (*Agaricus*) *mellea*, merely because the same disease in Europe was commonly attributed to attacks by these fungi. Underwood and Earle (*54, p. 272*), discussing root rot of the grapevine in Alabama, state that it seems to be identical with the disease known as "pourridié" in France. They believe that it is quite prevalent and often does serious damage, as evidence of which they mention the fact that out of the 584 vines in the station vineyard all but 83 showed evident signs of the disease, and that many died during the late summer and fall. They note that the character of the soil apparently has much to do with the prevalence of the disease, that vines may live many years while more or less affected by it, and that some varieties are evidently much more resistant than others. Earle and Austin (*14*), writing four years later, give a much more detailed account of grapevine root rot in Alabama and question the earlier statement of Underwood and Earle (*54, p. 272*) that this disease is the same as the "pourridié" of the French, which they say is caused by *Dematophora necatrix*. Repeated attempts to isolate and culture the organism causing the whitish mycelial growth under the bark of diseased roots were unsuccessful and further observations led Earle and Austin to conclude that the disease works much more slowly than the European root rot. These authors state that the mycelial whitening can often be found on old Scuppernong vines and on wild grapevines in the woods, although these are seldom if ever killed by it, and that its presence on the roots of cultivated vines is by no means a sure sign of immediate death. From an examination of the statistics on grape planting at the Alabama station these authors conclude that the disease is a very serious one. They state that of the 651 vines alive or planted in 1894, a loss of 483, or 75 per cent, was sustained in six years and, although there was no proof that all of these were affected by root rot, they believe that the greater part of them died from this cause. These authors conclude that the Herbemont and Rulander varieties are immune and that the Delaware is very resistant to mortality from root rot.

In 1901 Wilcox (*58*) described a rhizomorphic root rot of fruit trees which was causing widespread destruction of orchards in Oklahoma and adjacent States. He demonstrates that this disease is caused by a species of Clitocybe, which he describes as a new species, *Clitocybe parasitica*. In addition to its occurrence on fruit trees, he finds the fungus to be a common parasitic and saprophytic form on four species of oaks in Oklahoma. A rhizomorphic root rot, which Wilcox assumes was caused by this species of Clitocybe, is reported as occurring also in Texas, Missouri, southern Illinois, Indiana, and to some extent in Ohio, Georgia, California, and Oregon. Except for a few references in the bibliography appended, Wilcox makes no mention whatever of this disease on the grapevine, which, in some cases, undoubtedly is caused by the same fungus as that with which he was working.

Walker (*57, p. 30*) and Hewitt and Hayhurst (*21, p. 424*) reported that root rot of fruit trees is widespread in Arkansas and causes serious damage in some localities. The first author says that, "besides the apple it affects probably all of the commonly cultivated fruit trees, the grape as well as a number of forest trees," and states that the disease is caused by toadstool fungi, "two of the forms concerned being *Clitocybe parasitica* and *Armillaria mellea*."

Duggar (*13, p. 471*) mentioned the abundant occurrence of *Clitocybe parasitica* at Columbia, Mo., during favorable seasons on roots of hickory and other deciduous trees, but failed to observe its occurrence in orchards, despite special effort to find it. The suggestion by some observers that *Armillaria mellea* is responsible for the root rot of fruit trees attributed to *Clitocybe parasitica* is refuted by Duggar, who states that he has never detected this fungus associated with the typical disease in Missouri. Although *Armillaria mellea* may occur in Missouri, and in this case be responsible for some of the root rot reported, the writer has never seen it in the State even in forests where in favorable seasons *Clitocybe parasitica* often abounds.

Butler (*8, p. 24–29*), in his account of root rot of grapevines in California, describes a slow and a rapid form of

the disease, but contributes nothing to the identity of the causal organisms involved. The vineyard in which an exceptionally severe form of root rot was observed was planted on a slope shortly after the land had been cleared of its oak timber, and the lower part was poorly drained. From his experience with California conditions, the writer would attribute the form of root rot prevailing there to *Armillaria mellea*.

SYMPTOMS OF THE DISEASE

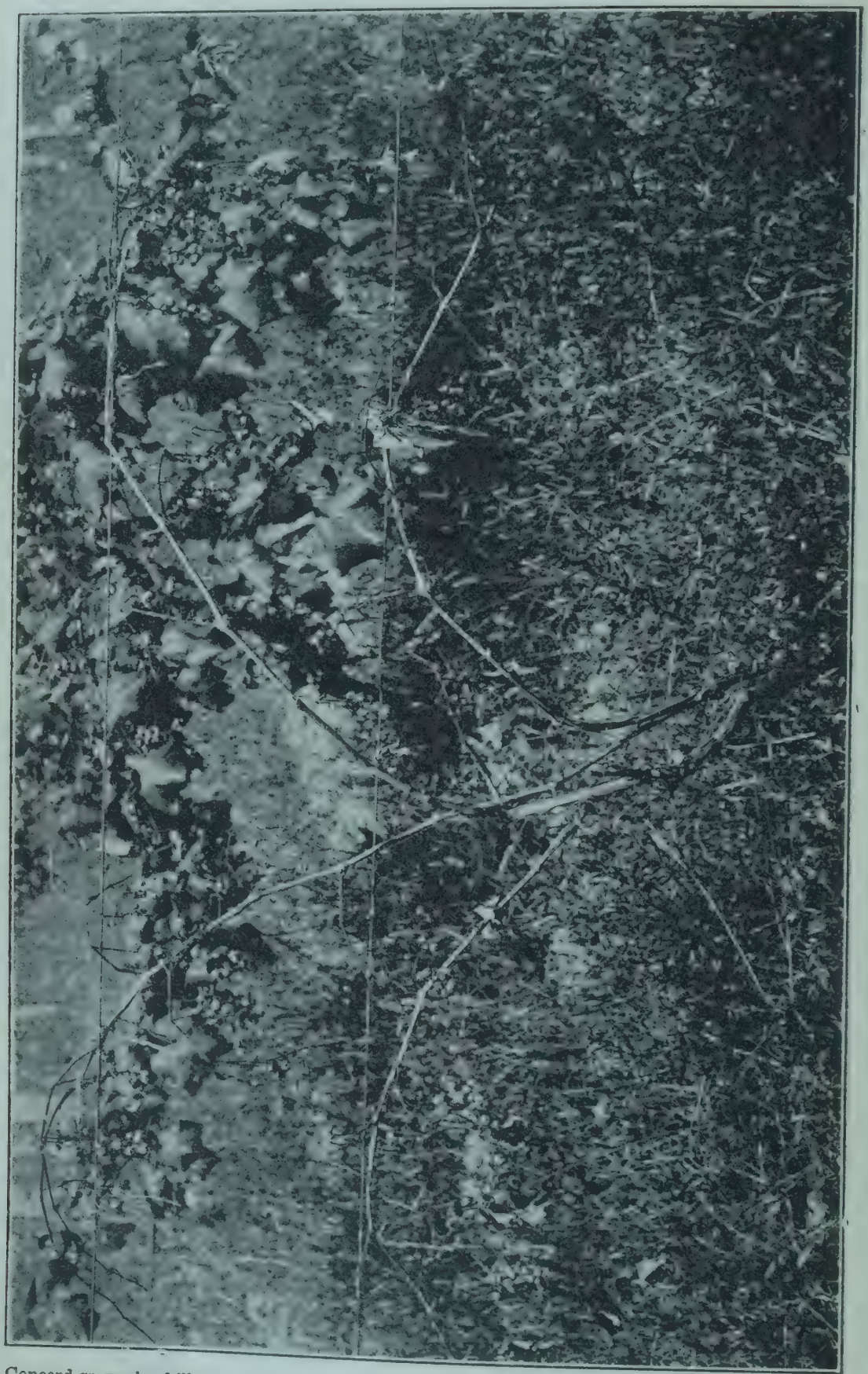
Often, without any cause apparent to the vineyardist, the vines will exhibit a sickly appearance which becomes quite evident in midsummer, when the demand for water conduction is greatest. At this time the margins of the leaves on diseased vines suddenly turn brown, usually shortly before the ripening of the fruit. Later the leaves may dry up entirely and the vine suddenly die, leaving the fruit to shrivel in the sun (pl. 1), or the crop may mature and the vine linger until fall, being entirely dead at the winter pruning. In other cases only a part of the vine will die, some branches putting forth a feeble growth for two or three seasons longer.

The root crowns and larger roots of diseased vines, when dug up and the outer fibrous bark peeled off, invariably show a whitish to creamy white or isabelline mycelial coating or sheet, the marginal portions of which spread out in a fan-shaped manner, the older parts often forming a feltlike layer of fungous tissue between the outer bark and the wood (pl. 2). Often there is present also a number of more or less flattened, black rhizomorphs. The inner living bark has been killed by the mycelium, which usually can be traced from 1 to 3 inches above the ground line and down to all the larger roots. In well-advanced cases the smaller fibrous roots may be rotted away, although in less advanced cases only the crown and the larger roots may be affected, the smaller ones remaining for the most part healthy. There is comparatively little decay of the wood of the root crown or larger roots until after the death of the vine, in which case transverse sections through the root crown or larger roots exhibit whitish radial streaks of more or less delignified wood.

The course and nature of the decay are essentially the same as those described by Wilcox (*58*) for fruit trees. Within the wood the mycelium attains its greatest development in the medullary rays, probably by reason of the fact that these are centers for the storage of reserve food materials. This great growth of mycelium within the larger medullary rays soon leads to the formation of radial cracks in the wood, which become stuffed with light tan-colored sheets or feltlike mats of mycelium often extending into the pith. This tendency of the mycelium to develop most abundantly within the larger medullary rays explains why the decay first appears as whitish more or less delignified radial streaks. Within the centers of decay the cell walls fail to respond to microchemical tests for lignin and various stages of dissolution are to be seen there. Within the individual cell the dissolution proceeds outward from the laminae bordering on the lumen, the middle lamella or primary layer being the last part to disappear. Contrary to the statement by Wilcox (*58, p. 15*) that the hyphae find their way into the cells only through the pits, the writer finds that they may penetrate the walls regardless of the presence of pits, as is the case in virtually all fungi causing an enzymatic digestion of the wood elements and the consequent decay of the wood.

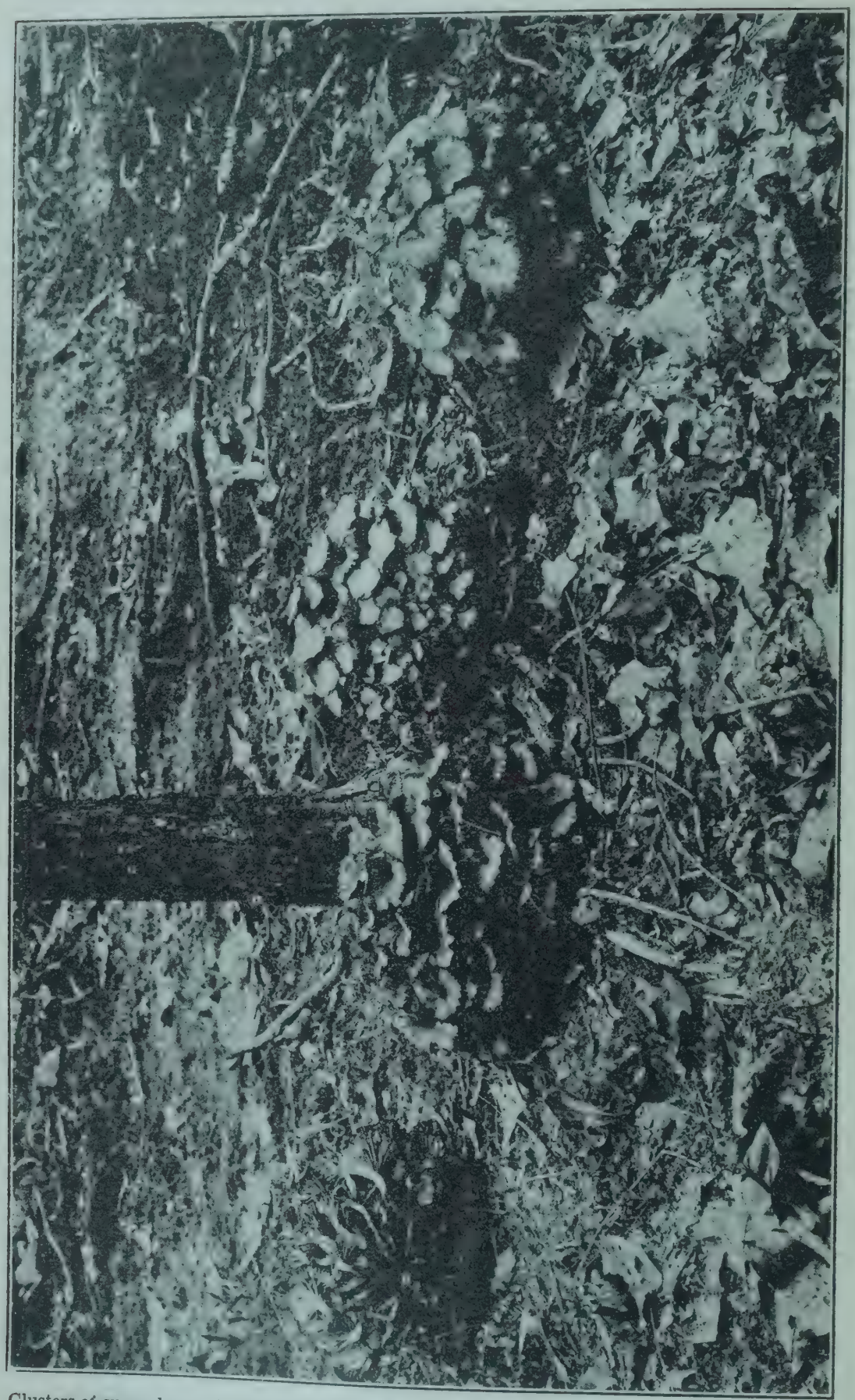
The mycelial growth through the inner bark is by no means confined to vines in which the foliage has shown signs of disease, but frequently may be found on those that are still making a fairly strong growth and on which the foliage is perfectly healthy. This point can be determined readily in an infected vineyard by making a slicing cut down through the outer bark at the ground line. The presence of the mycelium in the inner bark of the roots is by no means an indication of speedy death, however, for the disease works comparatively slowly and vines may be affected for a number of years before they succumb. Although the writer has no definite figures on this point, it is believed that at least from two to four years are required for this root rot fungus to kill a well-established vine. When the mycelium has progressed sufficiently through the wood and the inner living bark to cut off, either wholly or in part, the water supply, the vine dies more or less suddenly.

During the late summer and autumn, in favorable years, one often finds in vineyards and orchards suffering from root rot clusters of the mushrooms fruiting from the root crowns of vines and fruit trees. These may be found even more abundantly in forests, especially in oak stands (pl. 3).



Concord grapevine killed by Clitocybe root rot. All the leaves and fruit dried up shortly before the harvest. The living leaves showing in the background are on the vine in the next row

Rootstock of Concord vine recently killed by Clitocybe root rot. The outer fibrous bark has been peeled away to show the whitish to buff colored mycelial mat and the branching blackish rhizomorph at the point marked R. The level at the top of the picture was but a short distance below the ground line. Three-fourths natural size



Clusters of sporophores of *Clitocybe tabescens* in woods consisting largely of oak. Hundreds of these clusters of mushrooms occurred in one section of woods, most of them growing from the bases and roots of dead trees and stumps, or from buried wood, while a few others grew from the bases of living trees. The cluster at the left was placed in the field of view to show the character of the under side

CULTURAL STUDIES

Cultures made from the mycelial sheets on the roots of grapevines affected by root rot invariably yielded a very characteristic, slow-growing organism. Very scant growth of the fungus or none was obtained on cornmeal agar, and cultures on plain agar resulted in but weak growth. A much better growth was secured on prune agar and a very satisfactory growth on both maltose agar (30 gm. maltose per liter) and raisin agar (50 gm. seeded raisins per liter). The growth of the fungus on these different media indicates that it does best on media rich in sugar.

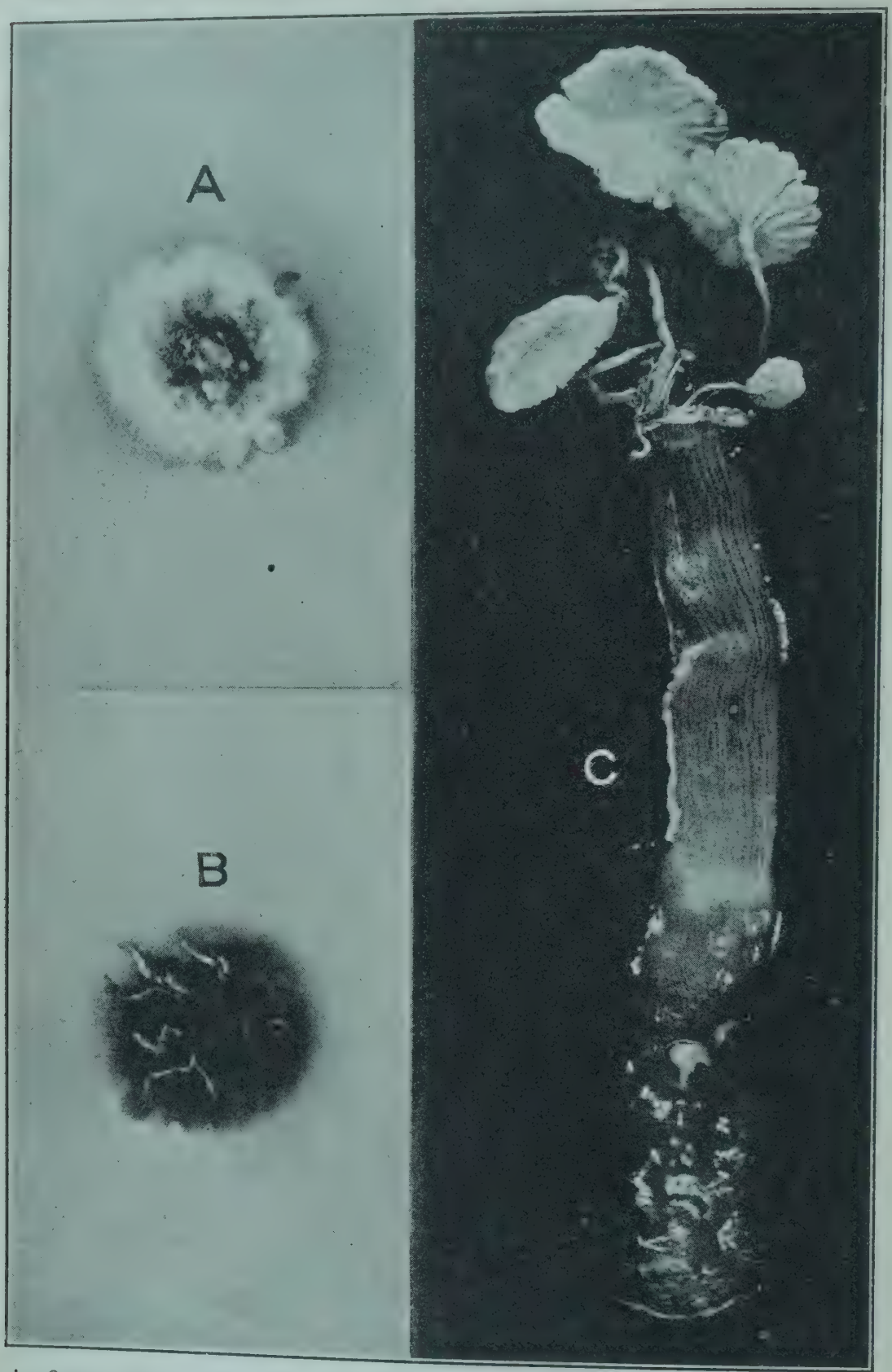
Since this organism develops rhizomorphs that grow down into the agar, it was found highly desirable early in the work to have the culture media as clear as possible in order to best observe the development and morphological characters of these structures. In order to clarify the agar, the whites of two eggs were added to each liter and the whole boiled prior to tubing and sterilization.

The initial mycelial growth was whitish, the marginal growth in most cases being rather sparse and downy-appressed, although in a few instances a rather dense white marginal growth occurred (pl. 4, A). In all cases the mycelial growth eventually became thickened at the center, so that it presented a convex outline, the older portion near the center soon becoming a dirty white and later changing to isabelline or light buff. Often the immediate central part would appear as an irregular, light tan-colored lump. In the case of cultures made on raisin agar the agar invariably became discolored, showing dark brown immediately beneath the mycelial mat and for some distance in advance of it. No such discoloration was noted, however, in any of the cultures on maltose agar. After the lapse of from two to three weeks, rarely longer, there would grow down into the agar, from the central part of the culture, peculiar, tortuous whitish strands, which constitute the beginning of rhizomorph formation (pl. 4, B). The plate cultures invariably dried up before the fungus attained any great development, so that their use was abandoned early in the work. Even tube cultures made in the size of tubes commonly used dried up as a rule before fruiting bodies developed. The best results with this slow-growing organism were obtained by making the cultures in large tubes and flasks. In some of the small-tube cultures a group of little hornlike processes—the primordia of clusters of mushrooms—often developed from the central part of the culture, but never developed further on account of the drying up of the cultures.

On July 19, 1922, the fungus was inoculated on 4-inch lengths of roots of wild grape (*Vitis cordifolia* Michx.), autoclaved in large test tubes containing a small quantity of water. From the mycelial transfer, which was placed on the top of the root length, the mycelium quickly grew down through the inner bark, even to the bases standing in water. At a few points it pierced the bark and developed superficial masses of mycelium, a few light tan-colored nodules forming on the tops.

Just a few days before the fruiting of one of these sets of cultures, the writer, while making a study of the grape diseases at Neosho, Mo., during the picking season, found several clusters of a caespitose species of mushroom developing from the bases of Moore's Early and Concord vines which had been killed by root rot in a badly infected vineyard. Large numbers of clusters of the same fungus were observed also in one section of an oak forest (pl. 2) several miles distant, growing from the bases of dead oak trees and from roots beneath the surface of the ground. One large cluster was also observed growing from the superficial roots of an old silver maple shade tree in Neosho. The morphological characters and spore measurements of these specimens agreed perfectly with those of *Clitocybe tabescens* (Scop.) Bres., of which Morgan's *Agaricus monadelphus*, Wilcox's *Clitocybe parasitica*, and Murrill's *Monadelphus caespitosus* represent the American forms. A representative specimen from both the vineyard and the oak forest was sent to Murrill, who identified both as *Monadelphus caespitosus*.

Upon the writer's return from Neosho he was agreeably surprised to find one of the series of cultures on the lengths of grape roots beginning to develop fruiting bodies. On August 10, a group of hornlike processes appeared on the top of the root length in one of the tubes. By August 17 this had developed into a miniature cluster of mushrooms that was reasonably typical of those collected in the field a few days before. With the lapse of four more days the mushrooms of this cluster had attained their full development (pl. 4, C) and had cast on the side of the tube a print of white



A.—One-month-old Petri dish culture of Clitocybe root rot, isolated from a recently killed vine, on raisin agar. The marginal mycelium is whiter and more superficial than in the case of cultures on other agars used. About 1¼ natural size

B.—Under side of same culture showing the dark-brown discoloration of the agar and the characteristic whitish rhizomorphs beginning to develop beneath the surface of the agar. About 1¼ natural size

C.—Cluster of sporophores of *Clitocybe tabescens* developed in artificial culture made by inoculating a sterilized length of root of *Vitis cordifolia* in a large test tube. This cluster of sporophores developed to maturity and cast a print of white spores on the side of the tube within one month and three days from the time the length of root was inoculated. About natural size

spores typical of *Clitocybe tabescens*. Thus mature fruiting bodies shedding spores were developed within approximately one month from a mycelial transfer.[5]

None of the other cultures of the series on lengths of wild grape roots fruited, some becoming contaminated with molds and others drying up. No rhizomorph formation was noted on any of the lengths of roots, but, when the bark was peeled off, all exhibited the whitish fan-shaped mycelial sheets characteristic of the root rot of grapevines found in the field. The source of the inoculum for this set of cultures was the rotted vine roots collected at Knobview in the early part of the summer, at which time no fruiting bodies were in evidence.

The type of the root rot, including the fan-shaped mycelial sheets and the rhizomorphs, of the vines at Neosho was identical with that of the Knobview vines first studied, and cultures secured from the rotted vine roots bearing sporophores at Neosho agreed in every way with those secured from the Knobview material. Although the writer has had no opportunity to attempt the reproduction of this root-rot disease of grapevines by inoculations with pure cultures of the fungus isolated from infected vines, he is thoroughly convinced that it is caused by this rhizomorph-producing fungus.

In this connection it is of interest to note that Knobview, where the writer obtained his first cultures of the grape root-rot fungus, from which material he succeeded in rearing the fruiting bodies of *Clitocybe tabescens*, is but about 70 miles by air line from Bushberg, where Scribner (*46, p. 137*) reported the discovery of grape root rot in this country in 1887. The evidence at hand strongly indicates that the organism isolated by the writer, which causes a more or less serious root rot of grapevines in many sections of southern Missouri, was likewise the cause of the root rot reported 38 years ago, rather than *Armillaria mellea*, as is generally believed according to Piper and Fletcher (*39, p. 7*) and Hesler and Whetzel (*20, p. 97*).

On June 12, 1923, a series of cultures in Erlenmeyer flasks was begun with a view to observing the production of rhizomorphs and fruiting bodies where a large quantity of the substratum and a larger space for growth were provided. Six cultures were made in 500 c. c. flasks containing a quantity of small pieces of roots of a wild grapevine (*Vitis aestivalis* Michx.). Twelve other cultures were made in 150 c. c. flasks filled to a depth of three-quarters of an inch with agar. Six of these contained maltose agar, the other six raisin agar. One of each of these three sets of six flasks was inoculated with a pure culture of the Florida form causing root rot of eucalyptus, which was kindly furnished by Miss C. Audrey Richards, of the Madison branch of the Office of Investigations in Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture. The remaining five flasks in each of the three sets of six were inoculated with pure cultures of the grapevine root-rot fungus secured by the writer at Knobview, Mo.

All the cultures made a slow but steady growth after the lapse of a few days. The different cultures presented certain minor variations in the appearance of the superficial mycelium, but no greater difference appeared between the two root-rot fungi than occurred in any one group of the three different substrata. The cultures all exhibited the same general appearance in so far as the aerial mycelium was concerned. The marginal growth, as a rule, was rather sparse and downy-appressed. At the center of the cultures the mycelium developed into thickened compact masses which sometimes were quite nodular at first. In some instances the downy-appressed marginal growth was lacking, the feltlike mycelial growth being thickened and convex clear up to the margin. The mycelium was white at first, but soon turned to a dirty white, later changing to isabelline or light buff, and eventually becoming light tan in the older portions.

After the lapse of from ten days to two weeks it was noted that in all of the six cultures on raisin agar the agar had become strikingly discolored, becoming dark brown in advance of the mycelium, whereas such a discoloration did not occur in any of the cultures on maltose agar. When but a

[5] In this connection it is of interest to note that Totten (*53*) reported cultural studies of this fungus (as *Clitocybe cespitosa*), but made no mention of the production of fruiting bodies. A report of the writer's successful rearing of the sporophores of this fungus was given in the following: RHOADS, A. S. CLITOCYBE PARASITICA AS A CAUSE OF ROOT ROT OF GRAPEVINES. Paper presented at 17th Annual Meeting, Bot. Soc. Amer. (Mycol. Sect.), Cambridge, Mass., December, 1922. [Not published. Title in program, p. 13.] On the same program there also appeared an abstract of a paper by Miss C. Audrey Richards (*41*) in which report was made of the rearing of sporophores of the latter fungus isolated from a piece of rotted eucalyptus root sent from Florida. *Clitocybe monadelpha* is but one of the several synonyms of *Clitocybe tabescens*, the discussion of the history and nomenclature of which will be deferred until later. These joint reports constitute the first record of the development of sporophores of this fungus in artificial cultures.

week old the cultures of eucalyptus root rot, on both maltose and raisin agars, quickly developed a number of light-brown branching rhizomorphs with whitish tips. These extended downward and outward from the central portion of the mycelial growth. The appearance of these structures was somewhat varied, but perhaps can be described best as at first antlerlike, closely resembling the branches of certain Clavarias. In some cases the ends were pointed; in others they were blunt and flattened.

When the cultures of the eucalyptus root-rot fungus were 2 weeks old, it was noticed that in both those on maltose and on raisin agar the ends of some of the rhizomorphs had turned upward and had continued growth in this position until they reached slightly above the surface of the agar. That the upward growth of these submerged organs was the result of their own volition and that they were not deflected upward by the walls of the flasks were clearly evident. The exposed ends were whitish and at their junction with the surface of the agar there soon developed outward a downy-appressed mycelial growth. In this way the growth of the fungus might be started anew at several points on the surface of the agar in advance of the superficial mycelium developed outward from the original point of inoculation. After reaching the surface of the agar the rhizomorphs grew but little longer. They rarely protruded above the surface of the agar more than from one-sixteenth to one-eighth inch.

With the increased age of the cultures the rhizomorphs gradually acquired a darker coloring until they became dark brown. When about 4 weeks old, it was noted on viewing the cultures by transmitted light that there radiated from many of the rhizomorphs a dense but delicate growth of whitish mycelium having an opalescent appearance, like the growth of certain bacteria in stab cultures. So dense was this halo of radiating mycelial growth that its filamentous character could be distinguished only at the periphery, even with a hand lens. Beginning at the ends of the rhizomorphs, or practically so, this radiating halo of mycelium gradually became broader, proceeding away from the end until it had attained a length of as much as 5 mm., measured radially from the rhizomorphs. The appearance called to mind the root-hair development on a radish seedling, except for the fact that the radiating mycelial threads were much more minute and dense in comparison. Other rhizomorphs without this root hairlike development of delicate radiating mycelial filaments had numerous little white branches from 1 to 3 mm. long that developed out of the old brown rhizomorph at more or less of a right angle, like the aerial roots along the stem of the English ivy. Sometimes there would be one every millimeter or two, or a space of several millimeters might occur without them. In other cases these branches would develop in pairs or even in tufts of several, some of which were branched. Eventually these numerous, short, more or less radially disposed lateral branches of the rhizomorphs also became brown. This is truly a remarkable fungus that is well worth an intensive cultural study.

In a number of the cultures in this series, chiefly those of the Florida form, clusters of mushrooms made their appearance after a more or less definite period of time and attained a more or less perfect stage of development. The first evidence of sporophore formation was the development of a little group of hornlike processes which quickly differentiated into a cluster of embryonic mushrooms. The time at which fruiting began is based in the tables upon the appearance of the latter. In practically every case the clusters of fruiting bodies developed first from the central portion, or oldest part, of the culture. After one cluster of mushrooms had started its development at this point, from one to five others sometimes appeared, developing beside the first one, or at the periphery of the central mycelial mass, or at points farther out from the central mycelial mass, such as the ends of the rhizomorphs which had turned upward and reached the surface of the agar, or from the mycelial growth developed where the ends of the rhizomorphs appeared at the surface of the agar. As a rule, only the cluster of mushrooms first appearing attained full development, and only a few of the sporophores within this cluster attained any great size. In a few cases, however, a second, and even a third, cluster of mushrooms developed in more or less rapid succession. The caps were hemispherical at first, but quickly became more or less sharply centrally depressed or depressed-umbonate as they expanded to their full development. The young sporophores presented a considerable variation in color, ranging from a creamy white when very young to buff or fawn color as they became somewhat older. As they approached maturity the marginal portion of the pileus

became yellowish white to buff and the central part darker and often ornamented with brownish fibrils. The gills were pure white at first and distinctly decurrent. No evidence whatever of an annulus was seen. As a rule, about a week was required for the embryonic sporophores to develop to maturity and shed spores, after which they quickly became sodden and discolored. Occasionally, the development of the sporophores was checked, and they dried up before shedding spores.

The Missouri form causing the grapevine root rot behaved essentially the same in culture as did the eucalyptus form from Florida described in the four preceding paragraphs, differing only in that it gave a very meager and more delayed development of rhizomorphs as compared with the latter. The Missouri form likewise developed fruiting bodies in the cultures much more slowly and with much more difficulty than the Florida form. The development of these two forms of *Clitocybe tabescens* may be compared in Table I, which shows the length of time required for each to reach certain definite stages of development on three different substrata. Inasmuch as the writer left Missouri, the cultures were abandoned after the lapse of 110 days.

The fruiting bodies which the writer succeeded in developing from the cultures of the Missouri form in this series of cultures and in other miscellaneous ones were an exact counterpart of these developed by cultures of the Florida form, and the writer is convinced that these two forms of root rot represent simply two different strains of the same fungus, namely, *Clitocybe tabescens*. The more luxuriant development of rhizomorphs and stronger tendency to develop sporophores exhibited by the Florida form may possibly be an acquired environmental character.

On June 30, 1923, a series of cultures of the Florida form was started from basidiospores taken from a spore print cast on the side of the tube in which a group of mushrooms had developed in a former culture. This series was made with a view to determining the length of time required to run this fungus through its full life cycle from spore to spore and to compare this with the length of time required for mycelial transfers of the same form to produce fruiting bodies. Unfortunately, spores of the Missouri form were not available at this time, nor was the opportunity afforded the writer of collecting material in the field to furnish spores; so that no comparison can be made between the two forms of *Clitocybe tabescens* grown from the basidiospores.

The results of the experiment with the Florida form, presented in Table II, indicate that it makes no difference in the length of time required for this form to develop fruiting bodies,

TABLE I.—*Flask cultures for comparison of the Florida and Missouri strains of Clitocybe root rot*

Culture medium and number of culture	Source of organism	Number of days required for—		
		Appearance of rhizomorphs	Differentiation of sporophores	Maturation of sporophores
Pieces of *Vitis aestivalis* root:				
1	Eucalyptus from Florida	22	44	50
2	Grapevine from Missouri	15		
3	do	31	76	83
4	do	33		
5	do	37		
6	do	37		
Maltose agar:				
1	Eucalyptus from Florida	7	37	50
2	Grapevine from Missouri	22	75	86
3	do	35		
4	do	35	77	87
5	do	37		
6	do	37		
Raisin agar:				
1	Eucalyptus from Florida	7	36	42
2	Grapevine from Missouri	18		
3	do	18		
4	do	18		
5	do	21		
6	do	35		

whether the cultures are started from freshly cast basidiospores or mycelial transfers. In all cases the basidiospores quickly gave rise to a sparse, downy-appressed mycelium, the central part of which developed into a tough, convex, light tan mycelial mass, the surface of which was very finely and densely nodular. The marginal growth about this initial mycelial mass was a sparse, downy-appressed fawn to tan-colored mycelium. In their subsequent growth and behavior these cultures of the Florida form started from basidiospores (pl. 5) agreed exactly with those of the same form started from mycelial transfers.

TABLE II.—*Growth of the Florida strain of Clitocybe root rot in cultures made from basidiospores*

Culture medium and number of culture	Number of days required for—		
	Appearance of rhizomorphs	Differentiation of sporophores	Maturation of sporophores
Raisin agar (three small test tubes):			
1	7	31	37
2	7	31	37
3	8	32	38
Maltose agar (three large test tubes):			
1	7	31	38
2	8	31	38
3	9	31	38
Maltose agar, (three 150 c. c. flasks):			
1	8	33	38
2	9	34	41
3	9	36	43

HISTORY, NOMENCLATURE, AND GEOGRAPHIC DISTRIBUTION OF FUNGUS

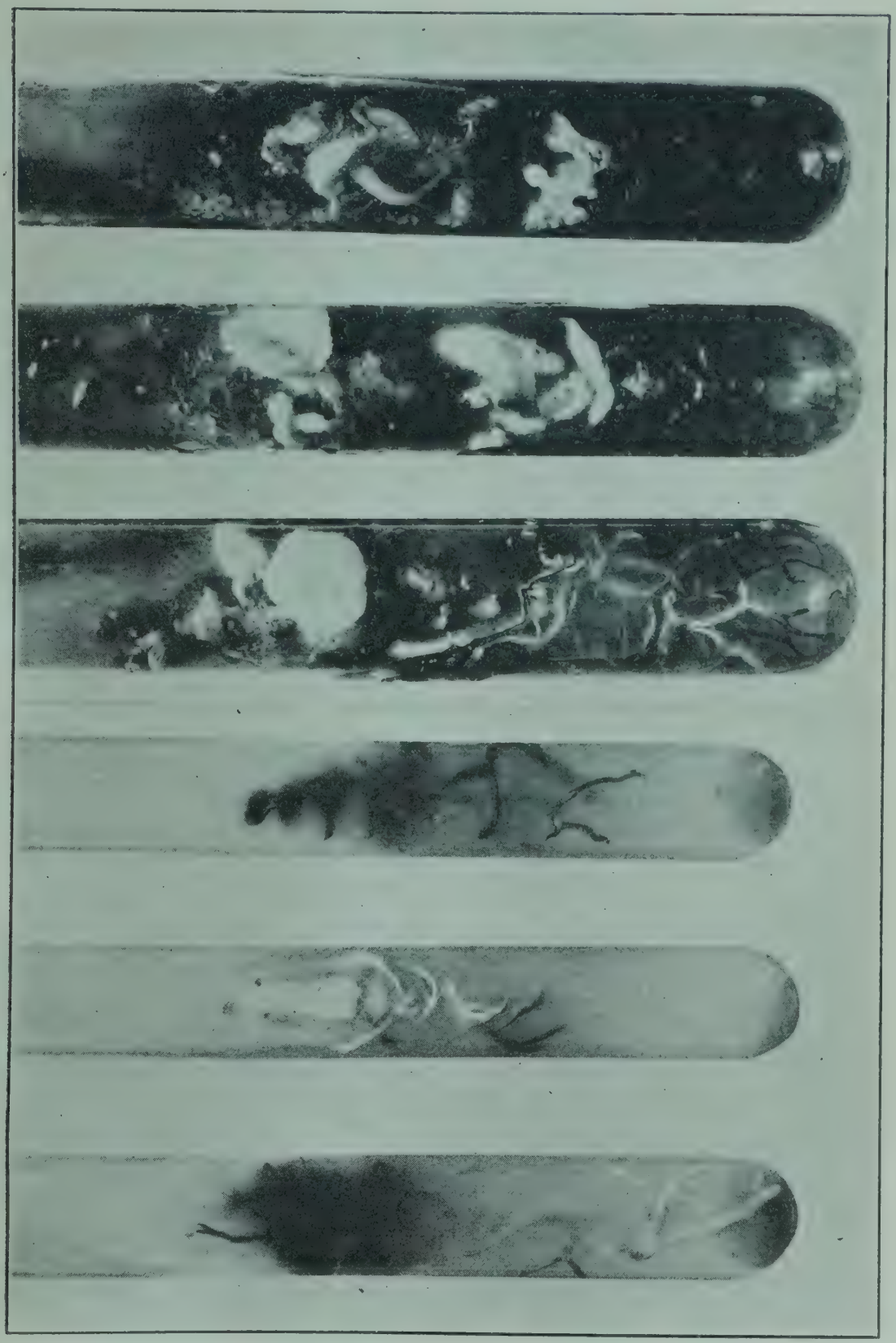
Early in the course of the investigation it became evident that although the species of Clitocybe causing the root rot of grapevines in Missouri was identical with Morgan's *Agaricus monadelphus* (*27, p. 69*), this was but one of several names that had been given to the same plant in the United States. Moreover, Bresadola's decision in 1900 (*6, p. 84–85*) that the plant which we have known best in the United States as *Clitocybe monadelpha* is the same as *Clitocybe tabescens* of Europe, which is accepted by practically all American mycologists who are familiar with this plant, adds still more to the multiplicity of names for it.

While various writers have from time to time given a partial list of the synonyms of this fungus, each has included but a small portion of the large total. The present writer, having made an extensive survey of the literature on this subject, has deemed it advisable and highly desirable, therefore, to review the history, nomenclature and geographic distribution of this fungus with the hope that its exact status and the relation of its numerous synonyms may be definitely settled and established.

Clitocybe tabescens (Scop.) Bres. is based upon Scopoli's description of *Agaricus tabescens* in 1772 (*45, p. 446*), Bresadola having decided that Scopoli's species represents the earliest authentic description of this much-named plant. Although the history of the plant in question appears perfectly clear since that date, the status of Scopoli's species and its relation to the species described by certain contemporaneous writers is somewhat of a mystery and probably will always remain so.

As a synonym of his *Agaricus tabescens*, Scopoli cites Haller's "Fungus siccior, pulvinatus, rufus, lamellis rarioribus," with the note "huic proximus," Scopoli's citation of the number of Haller's species, however, should have been 74 instead of 47. As synonyms of his species Haller (*19, p. 49, no. 74*) cites Micheli's "Fungus parvus, esculentus, odoratus, coriaceus, rufus, lamellis inter se longe distantibus" (*26, p. 148, no. 3*), with a question mark and the note, "Non repugnat," and Vaillant's "Fungus multiplex sordide carneus" (*55, p. 66, no. 36*), with the note, "Non recedit."

Agaricus gymnopodius illustrated by Bulliard (*7, pl. 601*) in 1780 and *A. socialis* described by De Candolle (*9, p. 48*) in 1815 are accepted by



Lower figure—Cultures of *Clitocybe tabescens* (Florida form causing root rot of Eucalyptus) developed on maltose agar 19 days after inoculation with basidiospores, showing the production of rhizomorphs. About natural size

Upper figure—Cultures of *Clitocybe tabescens* (Florida form causing root rot of Eucalyptus) developed on maltose agar 38 days after inoculation with basidiospores, showing the production of rhizomorphs and mature sporophores. About natural size

Bresadola (*6*) and others as synonyms of Scopoli's *A. tabescens*. *A. glomeratus* described and illustrated in 1824 by Pollini (*40, p. 679*), which, as he states, is an "*Agaricus gymnop.* aggregatus caespitosus saepius ramoso-connatus," etc., is not included as a synonym by Bresadola, although in Saccardo's Sylloge Fungorum (*43, p. 385*) it is included in the list of illustrations of *Clitocybe tabescens*, or *Collybia tabescens* as he calls it, following Fries. With allowances for poor drawing, Pollini's illustration might pass for a cluster of the sporophores of *Clitocybe tabescens* in which the bases of the stipes had anastomosed as a result of growth conditions.

Fries, who clearly had but little familarity with the plant or plants which we now know as *Clitocybe tabescens*, added greatly to the confusion. In his "Epicrisis" in 1836 to 1838 (*17*) he described, without having seen either of them, *Agaricus tabescens* under Tricholoma (*17, p. 33*) and *A. gymnopodius*, which he knew only from Bulliard's illustration, under Flammula (*17, p. 183*), assuming that the spores were colored because the gills were. As synonyms of *A. tabescens*, Fries cited Scopoli's description and illustrations by Battarra, Larber and Micheli, the latter being Micheli's Plate 74, Figure 2, on which Persoon based his *A. buxeus*. None of these illustrations cited by Fries bears the slightest resemblance to *Clitocybe tabescens* as understood by Bresadola. In a later work in 1874 (*18*) Fries exhibited no greater familiarity with these species, retaining *A. gymnodius* under Flammula (*18, p. 244*) and transferring *A. tabescens* to Collybia (*18, p. 111*), although uncertain as to whether or not the gills were decurrent and whether the spores were colorless or colored. His account of the latter species is especially confusing. After its description as No. 344 on page 111, in which he cites only Scopoli's description and *A. socialis* in the sense of De Candolle (*9*) and Seynes (*49*), he describes it again but differently in his appendix to Agaricus on page 319, again citing Scopoli's description but adding this time Micheli's Plate 74, Figure 2, on which Persoon (*37, p. 190*) based his *A. buxeus* in 1828. This illustration of Micheli represents his "Fungus pileolo desuper lacero," etc. (*26, p. 158*), which is a different species from that mentioned above as having been cited by Haller and upon which Scopoli based his *A. tabescens*. Since Fries does not cite the synonym which Scopoli gives in his description of *A. tabescens*, it appears that he disagrees with Scopoli's use of this as a basis of his species, for on page 704, under addenda, he says: "P. 319. *A. tabescens*. Hic diversus est a supra n. 344 citato. Utri synonymon Scopolii sit referendum, dubium." Even if this be so, the writer can not see any justification for Fries's making two kinds of *A. tabescens* out of Scopoli's brief description.

From the foregoing it is evident that there is considerable confusion and uncertainty as to just what species authors writing before Scopoli in 1772 considered as synonyms of his *Agaricus tabescens*. However, it is virtually of no taxonomic value, and is even futile from the standpoint of accuracy to attempt to trace this species back to the days before the existence of binomial nomenclature.

Owing to the universal acceptance of Fries's work, the views held by him have been perpetuated in subsequent writings. Thus, in Saccardo's "Sylloge Fungorum" (*43, p. 385*), which follows Fries, Micheli's figure of his "Fungus pileolo desuper lacero, etc.," is cited as an illustration of *Collybia tabescens*, as Saccardo calls it, although in the writer's opinion Micheli's figure does not even resemble the species. Persoon's *Agaricus buxeus* (*37*), which was based upon this illustrated species of Micheli's, is accepted as a synonym of *Agaricus* (*Collybia*) *tabescens* by Winter (*59, p. 852*), and doubtfully so in Saccardo's "Sylloge Fungorum" (*42, p. 12*).

In the middle of the nineteenth century the history of the American plant which we know to-day as *Clitocybe tabescens* began. It was collected first in Ohio by Lea, who sent it to Berkeley. In 1847 the latter published a description of it (*1*), calling it *Lentinus caespitosus*. In 1868, supplemented by additional specimens of the plant collected by Ravenel in South Carolina, it was renamed *Agaricus* (*Pleurotus*) *caespitosus* by Berkeley and Curtis (*2*), apparently on the advice of the latter, who stated that "this is certainly an agaric."

One year previous to this, however, Curtis (*11, p. 85*), in his catalogue of the indigenous and naturalized plants of North Carolina, lists "*Clitocybe caespitosus*, M. A. C." under Agaricus. Although Totten (*53*), on the strength of Curtis' citation, used the name *Clitocybe cespitosa* for the plant we now know as *Clitocybe tabescens*, Curtis's name must be regarded as a *nomen nudum*. Neither *Clitocybe caespitosa* Peck (*31, p. 61*) nor *Clitocybe caespitosa*

Pat. (*30, p. 248*) should be confused with Curtis's name, for they represent entirely different species.

In 1883, Schulzer von Muggenburg (*44, p. 256*) described as a new species a plant occurring in Hungary, Poland, and Transylvania, which he had had under observation for several years and regarded as an exannulate form of *Agaricus* (*Armillaria*) *melleus*, naming it *Agaricus* (*Collybia*) *inarmillatus*. This was regarded as a synonym of *Clitocybe tabescens* by Bresadola (*6*).

In the same year Morgan (*27*) described from Ohio a plant which closely resembled *Agaricus* (*Armillaria*) *melleus*, but which lacked an annulus. This he called *Agaricus monadelphus*, placing it under Clitocybe.

In 1892 Peck (*32, p. 180–181*) published Miss Banning's description of *Clitocybe aquatica*, based upon specimens from Maryland. A year later Peck (*33, p. 134*) under his description of *Armillaria mellea*, mentions the receipt from Brooklyn, N. Y., and Washington, D. C., of a densely caespitose, slender-stemmed form with no annulus, which he called var. *exannulata*. The following remark by him is of interest: "It is scarcely distinguishable from *Clitocybe aquatica* Banning, and *Clitocybe monadelpha* Morg., which, I suspect, will yet have to be referred to this species. According to Quelet, *Clitocybe socialis DC.*, and *Agaricus gymnopodius* Bull. also belong here."

In 1895 Peck (*34, p. 265*) described *Armillaria mellea* var. *exannulata* in greater detail. In 1898 he reported for the first time the occurrence of *Clitocybe monadelpha* in New York (*35, p. 284*) and described and illustrated the species (*35, p. 302–303, pl. 51, figs. 1 to 5*). In his report on the edible fungi of New York in 1900, Peck (*36*) reproduced the same description of *Clitocybe monadelpha*, the illustrations being changed somewhat.

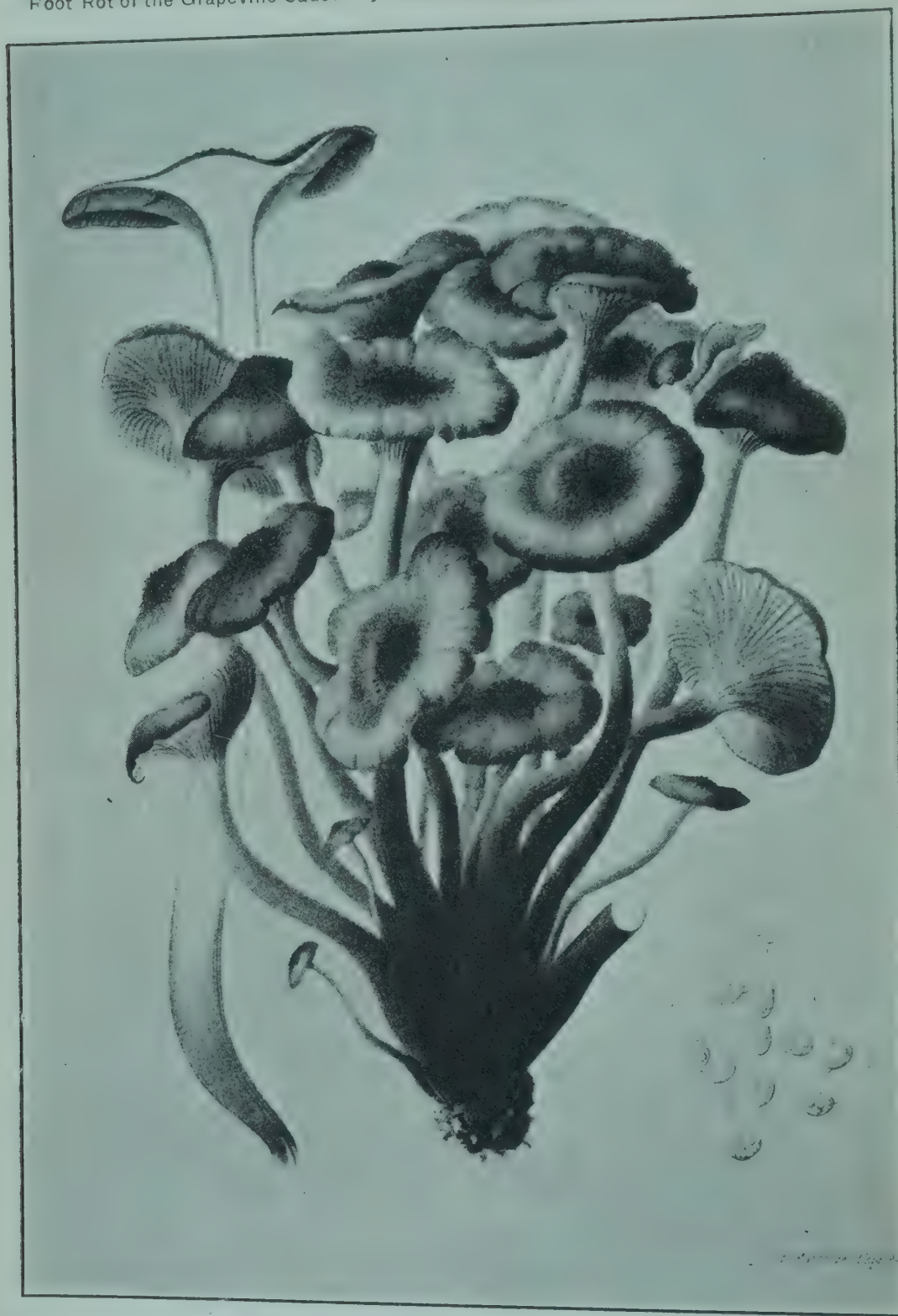
In 1900 appeared Bresadola's work (*6*), in which he pointed out that the plant we have known best in the United States as *Clitocybe monadelpha* is but a synonym of *Clitocybe tabescens* of Europe. It is generally conceded by most American mycologists familiar with this plant that Bresadola was correct in considering the American plant to be the same as the European. Boudier's excellent illustration, appearing as Plate 61 (51a) in his "Icones Mycologicae ou Iconographie des Champignons de France" and reproduced with slight modification as Plate 197 of Bresadola's work, is reproduced here as Plate 6. It is typical of our plants.

In his paper on a rhizomorphic root rot of fruit trees in Oklahoma caused by a caespitose species of Clitocybe, Wilcox (*58*) states that this species of Clitocybe is closely related to *C. monadelpha* Morgan and to *Armillaria mellea exannulata* Peck, but he describes it as a new species, *C. parasitica*, since he believed that it differed from these two species in certain morphological characters and in its parasitic habit of growth.

Unfortunately, the writer has not been able to see the type of Wilcox's species. Specimens do not occur either at the Missouri Botanical Garden, where he spent a few weeks in examination of the literature, or at the Oklahoma Agricultural and Mechanical College, where, if any were deposited, they probably were destroyed in the 1914 fire which consumed the herbarium and all records pertaining thereto.

Almost the only points in which Wilcox's description of his species differs from Morgan's are these: In Wilcox's plant the pileus is roughened with small scales from the first instead of being at first glabrous and then scaly; the stipes are never twisted, and it "is always parasitic in habit." According to the observations of the writer on the plants in both Missouri and Florida, Wilcox's morphological distinctions are based on rather variable characters. It is obvious that fungi never can be stereotyped creations and that mycologists must allow a certain amount of latitude in the species concept, which, in this case at least, is but arbitrarily defined. Citing a statement in one of Morgan's letters to him, written in 1901, that *C. monadelpha* is never a parasite, Wilcox reports (*58, p. 18*) that "our species is always parasitic in habit," and at the bottom of the same page further states that "the species grows in dense clusters from the crowns of living trees and the stumps of dead trees but is strictly a parasitic form in both cases." In his summary on page 22 he says: "The same fungus is a common parasitic and saprophytic form on four species of oak in Oklahoma." It is obvious, therefore, that neither the distinctions made by Wilcox in the morphological characters nor those in the habit of growth of these two fungi hold good.

Wilcox regarded the Oklahoma form as distinct from Peck's *Armillaria mella exannulata* because the plants



Reproduction of Boudier's illustration of *Clitocybe tabescens* given as Plate 61 (51a) in his "Icones Mycologicae ou Iconographie des Champignons de France," (5). Reduced to about ⅗ size of original plate

were larger and the caps not smooth, as Peck mentions in one place for his species, and because it never exhibited any evidence of an annulus even in the young stages, Peck's plant having occasionally had an evanescent annulus. These points of difference again clearly are variable characters and the writer is of the opinion that both plants should be included as synonyms of *Clitocybe tabescens* in the sense of Bresadola.

Murrill, in his monograph of the Agaricaceae of tropical North America (*28*) and in his monograph of the genera Clitocybe and Monadelphus in the North American Flora (*29, p. 420–421*), retains in Clitocybe the nonwood-loving caespitose species of Clitocybe and restricts the genus Monadelphus [6] founded by Earle (*15, p. 432*) to receive the caespitose species of Clitocybe, to include only those that are wood-loving.[7] Murrill makes no mention of *Clitocybe tabescens*, and Bresadola's determination that the plant we have known best in the United States as *Clitocybe monadelpha* is but a synonym of this species appears to have been overlooked entirely by him. The plant which is the subject of this paper is treated as *Monadelphus caespitosus*, based upon Berkeley's *Lentinus caespitosus*. Morgan's *Agaricus monadelphus*, Miss Banning's *Clitocybe aquatica* published by Peck, Peck's *Armillaria mellea exannulata*, and Wilcox's *Clitocybe parasitica* are all given as synonyms, although the latter is prefaced with a question mark.

Various other synonyms of *Clitocybe tabescens* not discussed here result from the raising of the older subgenera to full generic rank or the transfer of a species to various other genera. Taxonomic notes of historical interest are given on this species by Lloyd (*23, 24*). The treatment of this species by Kauffman (*22, p. 723*) and by Coker and Beardsley (*10, p. 106–107*) is also of interest.

There follows a chronological list of all the synonyms of *Clitocybe tabescens* in so far as they are known to the writer, those prefaced with a question mark being doubtful. Following this is a chronological list of all the illustrations of this species as far as they are known to the writer and believed to be correct, those prefaced with a question mark being doubtful.

SYNONOMY OF CLITOCYBE TABESCENS (SCOP.) BRES.

Agaricus tabescens Scop., Fl. Carn. ed. 2. 2: 446. 1772.

Agaricus gymnopodius Bull., Herb. Fr., Pl. 601, Fig. 1. 1793.

Agaricus socialis DC., Fl. Fr. 6: 48. 1815. Not *A. socialis* Fr., Ic. Hymen. 1: Pl. 49, Fig. 2. 1871; Hymen. Eur., p. 83. 1874.

?*Agaricus glomeratus* Pollini, Fl. Ver. 3: 679. 1824.

Lentinus caespitosus Berk., Lond. Jour. Bot. 6: 317. 1847.

Agaricus (*Pleurotus*) *caespitosus* Berk. & Curt., Jour. Linn. Soc. 10: 287. 1868.

Flammula gymnopodius (Bull.) Quél., Champ. Jura Vosg. 2: 346. 1873.

Clitocybe gymnopodia (Bull.) Gill., Champ. Fr., p. 162. 1874.

Agaricus (*Collybia*) *inarmillatus* Schulzer, Oesterr. Bot. Ztschr. 33: 256. 1883.

Agaricus monadelphus Morgan, Jour. Cinc. Soc. Nat. Hist. 6: 69. 1883.

Clitocybe monadelpha (Morgan) Sacc., Syll. Fung. 5: 164. 1887.

Collybia tabescens (Scop.) Sacc., Syll. Fung. 5: 206. 1887.

Pleurotus caespitosus (Berk. and Curt.) Sacc., Syll. Fung. 5: 352. 1887.

Clitocybe aquatica Banning and Peck, Ann. Rpt. N. Y. State Mus. 44: 180. 1892.

Armillaria mellea exannulata Peck, Ann. Rpt. N. Y. State Mus. 46: 134. 1893.

Clitocybe tabescens (Scop.) Bres., Fungi Trid. 2: 84. 1900.

Clitocybe parasitica Wilcox, Okla. Agr. Exp. Sta. Bul. 49: 18. 1901.

Monadelphus caespitosus (Berk.) Murrill, Mycologia 3: 192. 1911.

ILLUSTRATIONS OF CLITOCYBE TABESCENS (SCOP.) BRES.

Bulliard, Herb. Fr., Pl. 601, Fig. 1. 1793. (As *Agaricus gymnopodius.*)

?Pollini, Fl. Ver. 3: Pl. 2, Fig. 6. 1824. (As *Agaricus glomeratus.*)

Sicard, Hist. Nat. Champ., Pl. 31, Fig. 163. 1883. (As *Agaricus gymnopodius.*)

Morgan, Jour. Cinc. Soc. Nat. Hist. 6: Pl. 4. 1883. (As *Agaricus monadelphus*); also reproduced by Wilcox, Okla. Agr. Exp. Sta. Bul. 49: Pl. 6. 1901.

[6] It is of interest to note that this genus was given the variant spelling of "Monodelphus" by Earle in his generic description on page 432. This appears to be a typographic error, however, for it was spelled the usual way in his key on page 403.

[7] This is truly a superficial distinction for placing a group of species with the same general character in different genera. Even when collected carefully, it is not always clear whether these plants are growing from the ground alone or from buried wood, and, in case this point is not noted by the collector, the difficulty of making a correct determination of the species by these keys is increased.

Banning, Fungi of Maryland (MS. in Herbarium N. Y. State Mus.), Pl. 46. 1889. (As *Clitocybe aquatica.*)

Peck, Ann. Rpt. N. Y. State Mus. 51: Pl. 51, Figs. 1 to 5. 1898. (As *Clitocybe monadelpha.*)

Peck, Mem. N. Y. State Mus. 3: Pl. 46, Figs. 7 to 12. 1900. (As *Clitocybe monadelpha.*)

McIlvaine, Amer. Fungi, Pl. 27. 1900. (As *Clitocybe monadelpha.*) In Plate 16, Fig. 2, a poor illustration is given of Peck's *Armillaria mellea exannulata*, which is reproduced by Wilcox, Okla. Agr. Exp. Sta. Bul. 49: Pl. 4, Fig. 4. 1901.

Bresadola, Fungi Trid. 2: Pl. 197. (As *Clitocybe tabescens.*)

Wilcox, Okla. Agr. Exp. Sta. Bul. 49: Pls. 1 to 3, 5, 7 to 9; Figs. 10, 11, 14 to 16, 19. 1901. (As *Clitocybe parasitica.*)

Boudier, Ic. Myc. 1: Pl. 61 (51a). 1905–10. (As *Clitocybe tabescens.*)

Hard, Mushr., Pl. 12 (fig. 75). 1908. (As *Clitocybe monadelpha.*)

Duggar, Fungous Diseases of Plants, Fig. 234. 1909. (As *Clitocybe parasitica.*)

Rolland, Atlas, Pl. 25. 1910. (As *Clitocybe gymnopodia.*)

Coker and Beardslee, Jour. Elisha Mitchell Sci. Soc. 38: Pl. 12; 33, Fig. 7. 1922. (As *Clitocybe tabescens.*)

On his map in Plate 11, Wilcox (*58*) shows the localities in which occurs the rhizomorphic root rot described by him as found in Texas, Oklahoma, Missouri, Illinois, Indiana, Ohio, and Georgia, and adds that California and Oregon should have been marked as States from which the same disease had been reported. The rhizomorphic root rot reported by Wilcox from the two latter States, and assumed by him to be due to *Clitocybe parasitica*, was in all probability caused by *Armillaria mellea*, which the writer knows from personal observation is of widespread occurrence on the Pacific coast. So far as the writer's information extends, there never has been an authentic report of Clitocybe root rot occurring on the Pacific coast or in the Pacific Northwest.

From the reports of Peck we know that the species of Clitocybe that we now call *C. tabescens* occurs as far north as New York on the Atlantic coast. Kauffman (*22*) reported the occurrence of *Clitocybe monadelpha* in Michigan, and Fawcett (*16, p. lxvi*) reported *Clitocybe parasitica* as causing a root rot of peach in Florida. The cultures of Clitocybe root rot mentioned above as furnished by Miss Richards were also from Florida, having been isolated from a rotted eucalyptus root. Since coming to Florida in 1923 the writer has collected specimens of *Clitocybe tabescens* growing at the bases of stumps of *Washingtonia robusta* and *Ilex opaca* at Gainesville, and at the base of a living oak tree at Cocoa. He has also observed two instances of this fungus causing a root rot of guava (*Psidium guajàva*), one at Cocoa and the other near Courtenay on Merritt's Island.[8]

Murrill (*28, p. 193*) states that the species also occurs in Mexico and British Honduras. According to the taxonomic literature previously cited in this paper, the plant has a wide distribution in Europe. Nowhere in the European literature, however, has the writer seen any mention of its causing a rhizomorphic root rot of woody plants. In fact, even the cultural characters of the European form appear to be unknown.

In the United States the range of *Clitocybe tabescens*, which extends from New York south to Florida and west to Michigan, Kansas, and Texas, overlaps to a large extent the range of the closely related *Armillaria mellea*, which likewise causes a rhizomorphic root rot. Except for a few middle-eastern States, however, root rot caused by *Clitocybe tabescens* has been reported only from the southern part of the United States, where it is of most widespread occurrence.

RELATION OF CLITOCYBE ROOT ROT TO TIMBERED LANDS AND DRAINAGE

The root rot of grapevines and fruit trees caused by *Clitocybe tabescens* is associated with lands which were formerly covered with hardwood timber, especially oak. This disease appears to be practically unknown in strictly prairie soils and in old land, except at the margins in close proximity to timbered land. Men who have had no experience with the disease on old land have found that it quickly made

[8] Thus far the writer has not found grapevines attacked by Clitocybe root rot in Florida, but has had virtually no opportunity to investigate this problem since coming to this State. In a case of root rot observed on a single Herbemont vine at Bartow the fungus associated with the root rot did not appear to be Clitocybe nor did the one isolated prove to be a rhizomorph-producing fungus. Although cultures have been maintained for several months, neither spores nor fruiting-bodies have been secured.

its appearance upon extending their plantings into ground more recently cleared.

In all cases where the writer has had the opportunity to observe Clitocybe root rot, both of grapevines and fruit trees in Missouri, it has occurred in the low and poorly drained parts of vineyards and orchards where water is liable to stand in the soil, although it may also occur in soils underlaid with an impervious subsoil or hardpan, where natural drainage is poor. In Florida, however, Clitocybe root rot has been observed in sandy soils that appeared to be well drained. The relation of Clitocybe root rot to inadequate drainage was illustrated in very striking fashion in a vineyard about 18 years old at Neosho. This was planted in Moore's Early grapes on one side and Concord on the other. The land was practically level. Immediately adjoining one end of the vineyard was a sorghum field in which, close to the end of the vineyard, lay a large, slightly depressed area where water stood for long periods during the early summer. Even the sorghum in this vicinity had made but poor growth and appeared stunted and pallid. The grapevines at this end of the vineyard were weak and unthrifty. Large numbers had died out and been removed (pl. 7, upper figure), and still others were dying or were practically dead. Several of the dying vines were dug up and found to have the mycelium characteristic of Clitocybe root rot. Clusters of the mushrooms had even developed from the root crowns in some cases. In addition, it was determined by the cutting method previously referred to that many of the living vines which appeared to be at least moderately healthy were infected by the fungus. At the other end of the vineyard, which was 520 feet in length and contained but 52 vines, there was a full stand growing vigorously (pl. 7, lower figure).

The losses occasioned to vineyards by Clitocybe root rot vary greatly. In mild cases perhaps just a few vines will die from year to year, but in severer cases they may die quite rapidly until within a few years parts of the vineyard may be heavily depleted, as in the case just cited.

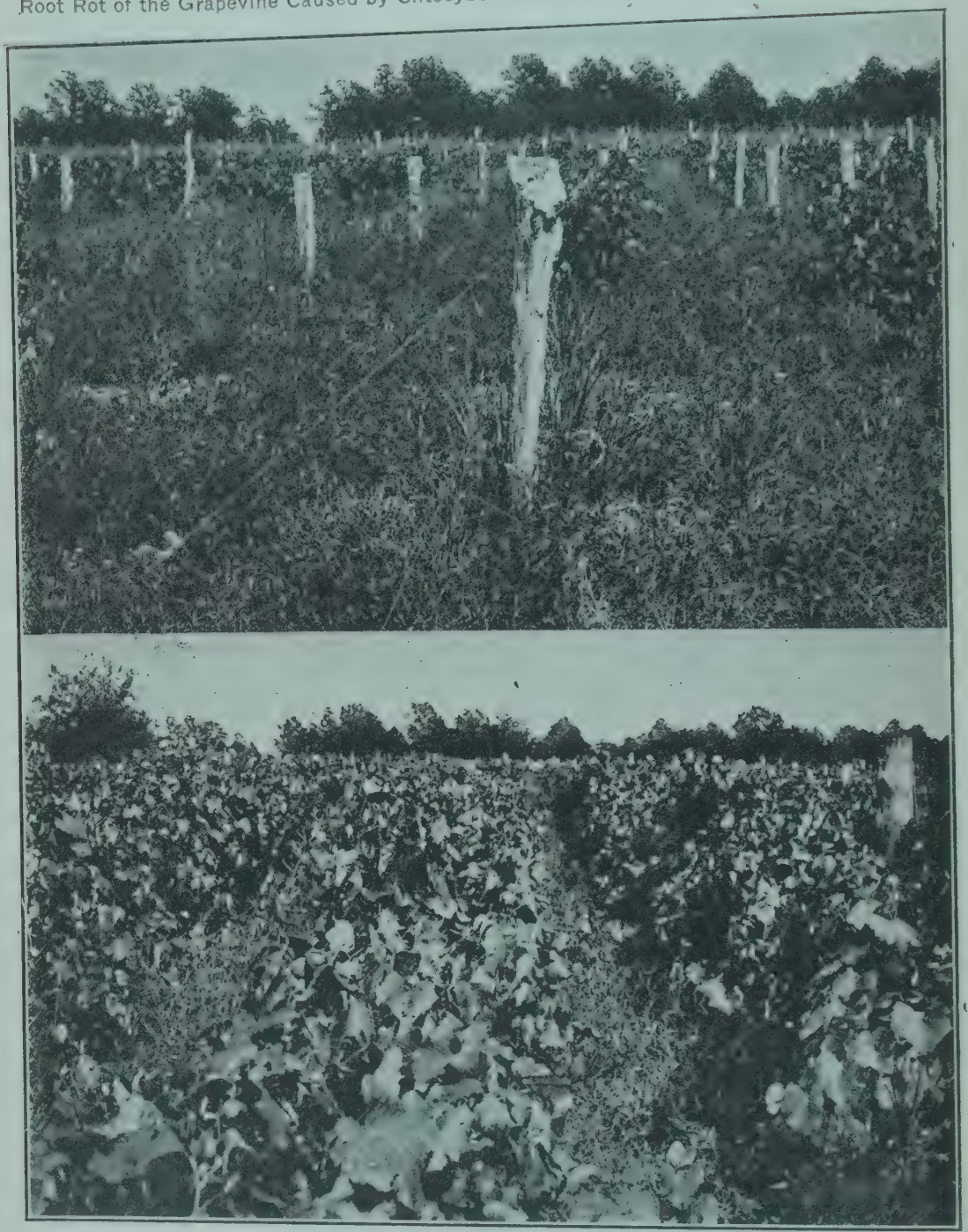
CONTROL OF CLITOCYBE ROOT ROT OF GRAPEVINES

A little attention directed to preventive measures will obviate the necessity of applying remedial measures in combating root rot which at best are but palliatives. In selecting a site for vineyard planting, newly cleared land, especially in a region of oak forests, should be avoided, since the fungus may live in the soil as a saprophyte on deadwood, such as stumps and roots. The character of the disease suggests the need for thorough preparation of cleared lands, including the removal of all roots, and cultivation for at least three years in other crops before setting out to grapevines or fruit trees. In any case, land which promises poor natural drainage should be avoided unless artificial drainage can be assured at a reasonable cost. Care should also be taken to prevent barking or otherwise injuring the roots of vines when cultivating the vineyard, since such injuries favor infection by root-rotting fungi. Control of the grapevine root borer is also essential for the same reason.

After Clitocybe root rot appears in a vineyard the vines that have been seriously attacked can not be saved; however, measures can be taken to check the spread of the disease and to save those recently attacked. Inasmuch as an excess of moisture in the soil, either as a result of a natural depression or being underlaid by an impervious substratum, is one of the chief conditions favoring the development of Clitocybe root rot, adequate drainage is, and will always be, the most valuable preventive and corrective measure. Unless this precaution is taken all others are useless, for vineyards that have become infected with this root-rot fungus are, unless drained, always subject to attack by it. The low or otherwise poorly drained places in the vineyard where water is liable to stand in the soil should receive most careful attention in this respect, for it is at these points that root rot is most likely to occur. As soon as the root rot is discovered in a vineyard all vines that have been killed by it and all vines badly diseased, that is, those whose scanty growth shows that their root system is seriously attacked, should be removed. These should be excavated carefully, care being taken to remove all the roots, which should be burned. Then, if the hole is left exposed to the air and sun for a few weeks or disinfection of the soil is accomplished by the use of chemicals, and the drainage of the soil has been assured, it should be safe to replant.

In European practice a number of chemicals have been applied to the soil to destroy the mycelium and spores of *Dematophora necatrix* and other root-rot fungi in the soil or to destroy the fungus on recently infected vines with-

Root Rot of the Grapevine Caused by Clitocybe Tabescens.



(Above).—Depletion of Concord vines by Clitocybe root rot in poorly drained end of vineyard. These vines continue to die out from year to year

(Below).—Full stand of thrifty Concord vines in well-drained end of vineyard shown above, the undrained end being in the background. The rows are 520 feet in length and contain only 52 vines. The transition between the sickly vines at one end and the vigorous ones at the other was rather abrupt

out injuring the vine itself. Bonjour (*4*) recommends the use of a 7 per cent solution of green vitriol (ferrous sulphate). Beniling and Behaix likewise recommend green vitriol against rot of vine roots where carbon bisulphide and sodium fluoride had no action. Dufour (*12*), however, who has conducted experiments to control pourridié of the grapevine for a number of years, tested comparatively the action of green vitriol and blue vitriol on the causal fungus (*Dematophora necatrix*) and found the latter much superior. He recommends a 3 per cent solution of blue vitriol (bluestone or copper sulphate) as a soil disinfectant. This is applied by excavating a small basin around the trunk of the vine and pouring into it a gallon or so of the solution, the exact quantity being determined by the nature of the soil and the supposed extent of the root system. Before planting new vines, 1 to 2 quarts of the same solution are poured into the holes. Vines adjoining those known to be attacked are also laid bare in the spring and watered freely with the solution; 3 to 5 ounces of granulated bluestone per stock may likewise be spread over the ground. The result is not always completely visible the first year, and it is sometimes necessary to repeat the treatment for two years in succession, in which case the results obtained are claimed to be very positive.

Dufour likewise tried ammonium sulphide, hyposulphite of soda and sulphite of lime against *Dematophora necatrix* without securing any promising results. Potassium sulphocarbonate, which Dufour and Mouillefert tried, also proved disappointing. Narbonne experimented with sulphur and advises laying bare the less badly attacked stocks as deep as possible and dusting the roots abundantly with sulphur, repeating the dusting several times before the stocks are covered again.

Carbon bisulphide has been tested extensively by numerous investigators for the control of insects and root rot in vineyards. Dufour made applications at the rate of 200 gm. per square meter, after having removed the diseased roots. According to this eminent investigator, carbon bisulphide destroys the mycelium of the fungus to a large extent. Blunno (*3*), experimenting later in Australia, states that carbon bisulphide applied at the rate of 1 ounce per vine, divided into five partial injections made within 6 to 8 inches from the stem, was effective in killing the mycelium living externally, that is, during the first stage of infection. Such a dose, however, was not sufficient to destroy the old foci of infection represented by the roots of the once existing trees, and repeated and stronger doses endangered the vines. According to the same author, ferrous sulphate applied each winter for three successive years at the rate of 8, 16, and 16 ounces, respectively, to each vine was sufficient to restore all but two vines in which the root rot yielded to a further and slightly modified treatment consisting of daubing the infected part of the stem with a solution made up of 10 per cent by weight of sulphuric acid and 50 per cent of ferrous sulphate. The ferrous sulphate was used in preference to copper sulphate because it was much cheaper. It was applied in the usual way by digging a hole around the stem 6 inches deep with a radius of 12 inches, at the bottom of which the chemical was scattered and the soil then replaced. This salt was thought to act not only as a fungicide but also as a vigorous stimulant to growth.

Szigethi-Gyula (*52*) states that experiments in a vineyard located in a rather moist situation showed that the application of lime at the rate of 2 kg., or 10 liters of milk of lime, to a vine was efficient in preventing pourridié.

Inasmuch as the mycelium of the root-rot fungi is often more resistant to toxic substances than the host plant, considerable experimentation will be necessary before there will be found for the control of root-rot fungi an efficient and satisfactory fungicide that can be recommended for general use with safety to the vine. It seems to the writer that paradichlorobenzene, which has proved so successful in the control of the peach-tree borer, would be well worth a trial for the control of root-rot fungi. So far as is known, this chemical is not especially injurious to grapevines. The above-mentioned fungicides are not recommended for general use, but are merely suggested for those who may wish to experiment in a small way with them.

The comparatively simple method of aeration and exposure to sunlight also has shown encouraging results in the control of root rot, both on grapevines in Europe and on fruit trees in this country. In this method the dirt is carefully excavated from about the root crown and larger roots and these are left exposed to the sun during the remainder of the summer, either with or without the application of a fungicidal wash. The soil is replaced before the advent of winter.

SUMMARY

A mushroom root rot of grapevines, of rather common occurrence in various localities in the Ozark section of Missouri, is reported and described in detail. The organism isolated from the roots of the diseased vines proved to be a very slow-growing but distinctive one, developing characteristic submerged rhizomorphs in the cultures. Fruiting bodies, while not associated with the diseased vines first studied in the field, were subsequently developed in some of the cultures. But a few days before the first fruiting bodies were secured in the cultures a species of Clitocybe was observed fruiting abundantly at the bases of rotted grapevines in a vineyard in another section of the State and in an oak forest a few miles distant. Cultures of the fungus isolated from the roots of these vines yielded a rhizomorph-producing organism identical with the one isolated from the first material studied. Although no opportunity of reproducing this root-rot disease by inoculations with pure cultures of the fungus isolated from infected vines has been afforded, it is believed that beyond all doubt the disease in question is caused by this rhizomorph-producing organism.

The fungus which is believed to be the causal organism is a species of Clitocybe described by various writers in this country under the names *C. monadelpha, C. aquatica, Armillaria mellea exannulata,* and *C. parasitica,* all of which are clearly different names for the same plant and identical with *Clitocybe tabescens* of Europe. A detailed discussion is given of the history, nomenclature, and geographic distribution of this fungus, together with complete lists of the synonyms and illustrations believed to be authentic.

A detailed description is given of the characteristics and behavior of the Clitocybe root-rot fungus in pure cultures and a comparison is made between the form isolated from grapevine roots in Missouri and one secured from a eucalyptus root from Florida. Although the writer's cultures of the Missouri form of Clitocybe root rot were generally much slower in their development and in the production of fruiting bodies than the Florida form, the two agreed so closely in their morphological characters that he is of the opinion that these two forms represent but two rather widely differing strains of the same fungus.

Although *Clitocybe tabescens* in the United States is known to occur from New York south to Florida and west to Michigan, Kansas, and Texas, cases of root rot caused by this fungus, with the exception of those in a few middle eastern States, have been reported only from the southern part of the country, where it appears to be of most widespread occurrence. The losses occasioned to vineyards in Missouri by Clitocybe root rot, which appears to be a comparatively slow-working disease, vary from the death of a few vines each year in cases of mild attack to such rapid destruction that within a few years parts of the vineyard may be heavily depleted.

Clitocybe root rot is a disease associated with lands which formerly were covered by hardwood timber, especially oak. The disease appears to be practically unknown in strictly prairie soils and in old land, except at the margins in close proximity to timbered lands. It has been found to attack grapevines and fruit trees chiefly in places where the soil is poorly drained, either as the result of natural depressions in contour or in places underlaid with a more or less impervious soil or hardpan.

Attention directed in the selection of vineyard sites to the thorough preparation of newly cleared timberlands, adequate drainage of any spots in need of it, and prevention of barking or other injury of the vine roots when cultivating the vineyard, will prove valuable measures in preventing attack by Clitocybe root rot. In case the disease is observed in vineyards already established, adequate drainage, which is and will always remain the most valuable remedial measure, should be assured, and the spread of the disease may then be checked by the prompt removal of all badly diseased and dead vines, the chemical treatment of adjacent vines, and the disinfection of the soil before replanting. The various methods for the destruction of the fungus on newly infected vines and in the soil are briefly reviewed and the need for further experimentation is pointed out.

Comparatively little study has been made of the fungi causing root rot of grapevines in this country. Although cases of this disease have been reported in various sections since its discovery in Missouri in 1887, it has, with few exceptions, been attributed to *Armillaria mellea* and *Dematophora necatrix,* as a rule on assumption, however, rather than from definite knowledge.

The present investigation affords very definite evidence that Clitocybe root rot, which hitherto has been certainly known to attack only fruit, forest, and shade trees, is responsible for a considerable quantity of root rot of grapevines, at least in Missouri, and probably also in other southern States. The isolation of this fungus by the writer from the roots of recently killed vines but 70 miles from the point where root rot of the grapevine was first discovered in this country indicates that *Clitocybe tabescens* was the cause of the case first reported rather than *Armillaria mellea*, as is generally believed.

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CONTROL OF MYCELIAL NECK ROT OF ONION BY ARTIFICIAL CURING[1]

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INTRODUCTION

Neck rot of onion, though widespread in its occurrence and at times very destructive, is very sporadic in its appearance. It has long been attributed to one or another species of Botrytis, but not until the work of Munn[3] upon *Botrytis allii* was a distinct species unquestionably established as a cause. Further study by the writer has shown that two other species of the genus are capable of invading the neck tissues of the onion and causing symptoms sufficiently similar to those caused by *B. allii* to create confusion with the latter. Since the comparative morphology of the three species and the symptomatology of the respective diseases are to be described in a separate paper, those phases will not be taken up in detail here. For convenience the three types of decay are designated as gray mold neck rot (*Botrytis allii* Munn), mycelial neck rot (*Botrytis sp. 110*), and small sclerotial neck rot (*Botrytis sp. 108a*). During the past nine years the mycelial neck rot has been by far the most destructive in the Racine, Wis., and Chicago, Ill., onion-growing sections. *Botrytis allii* sometimes occurs, but is not generally common; *Botrytis sp. 108a* is sometimes common on white varieties, but the nature of its attack does not make it as important as *Botrytis sp. 110*. Since the control experiments to be reported herewith were conducted in the Racine district, the results pertain entirely to the mycelial neck rot.

The severity of mycelial neck rot varies widely from year to year. Infection occurs presumably about harvest time. It has been supposed that the common practice of cutting the tops at this time exposes succulent wounds to the wind-borne Botrytis spores and thus favors infection. Controlled experiments to be described later show, however, that under certain conditions as much infection may occur with the white varieties in "untopped" as in "topped" bulbs. There is little doubt that climatic variation from year to year is a very potent factor in determining the amount of infection which occurs. Moist weather at the proper time to promote sporulation of the causal organism on dead refuse and to facilitate infection of the bulbs at the harvest period is apparently necessary to cause an epiphytotic. The signs of the disease appear one to three weeks later, first, as softening of the neck tissue, followed by the appearance of gray mycelial felt upon the decayed parts. From this time the fungus advances quite rapidly, causing collapse of the tissue, and in addition to superficial closely wefted gray mycelium there develop numerous black kernel-like sclerotia, while under sufficiently high humidity a gray to dark-brown layer of conidia is produced.

It has long been generally known among onion growers that white varieties of onion are more subject to neck rot than are the red or yellow types. Munn[3] has pointed out this difference in susceptibility to *Botrytis allii*. It is equally true for the mycelial neck rot and the small sclerotial neck rot. A discussion of the nature of resistance to onion neck rot has been given in a previous paper.[4] The serious losses are therefore usually in the white varieties, and it is with these that the problem of control becomes acute. In the two sections mentioned, the White Globe is the most common white variety grown for large bulbs, while the White Portugal is grown extensively for "bottom sets."

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[1] Received for publication May 29, 1924; issued April, 1925.

[2] Investigations conducted in cooperation with the Department of Plant Pathology, University of Wisconsin.

[3] MUNN, M. T. NECK ROT DISEASE OF ONIONS. N. Y. State Agr. Exp. Sta. Bul. 437, p. 361–455, illus. 1917.

[4] WALKER, J. C., and LINDEGREN, C. C. FURTHER STUDIES ON THE RELATION OF ONION SCALE PIGMENTATION TO DISEASE RESISTANCE. Jour. Agr. Research 29: 507–514. 1924.

SCOPE OF THE INVESTIGATION

The experience of the average onion grower has led him to recognize that prompt and thorough drying or curing of the bulbs, and especially of the neck tissue, is the most practicable means of reducing neck-rot infection. In ordinary practice this condition is approached by taking advantage of clear, bright weather and natural air currents and by affording shelter during periods of rain or high humidity. However, it is not always possible to avoid the disease by these means. The purpose of the present investigation has been to determine the effect of artificial drying of bulbs after harvest upon infection, and the feasibility of this as a practicable remedial measure. As a corollary to the above, a study was made of relation of maturity of tops and of the removal of the same at harvest time to the amount of infection which occurs. All of the experiments reported, with one exception, were performed on onions grown in the Racine-Kenosha district of southeasten Wisconsin.

EXPERIMENTAL RESULTS

RELATION OF MATURITY OF BULBS TO INFECTION

Methods of handling the onion crop vary in different localities. In some the bulbs are pulled and the tops clipped when the neck tissue is still quite succulent; in others, the tops are allowed to desiccate appreciably before they are removed; while in still others the tops remain attached for some weeks or months of storage. It is generally supposed that, other conditions being equal, the immature succulents necks are the most favorable for the beginnings of neck rot. Some observations on this were made at Racine in 1917 and 1918.

In each year a portion of a field of Red Globe onions was selected in which the plants were maturing unevenly. When the tops of the more advanced plants were thoroughly dried down, all of the bulbs were pulled and topped in the usual manner. They were then separated into two lots, which were designated "mature" and "immature" according to condition of the neck tissue. This was done on September 17, 1917, and on September 16, 1918. In the latter year a portion of both mature and immature bulbs were clipped so as to leave about one-half inch of neck tissue, while in the remaining portion 2 to 3 inches of neck was left. The bulbs were placed in 1-bushel folding crates and, according to the customary practice, were then stacked in the field to cure for about three weeks. They were then removed to an onion warehouse for storage. The bulbs harvested in 1917 were finally examined on January 15, 1918; those harvested in 1918 were finally examined on March 8, 1919. The results are given in Table I.

TABLE I.—*Percentage of mycelial neck rot developing in bulbs harvested with "mature" and "immature" neck tissue; Red Globe variety, grown at Racine, Wis., 1917 and 1918.*

Year and condition of neck tissue at harvest time	Length of neck left upon topping	Crate No.	Total number of bulbs included	Per cent of bulbs in which neck rot developed in subsequent storage
1917	*Inches*			
Immature	1	1	300	7
		2	300	6
Mature	1	3	300	3
		4	300	3
1918				
Immature	½	1	88	22
		2	91	23
	2 to 3	3	90	18
		4	84	23
Mature	½	5	163	11
		6	206	7
	2 to 3	7	127	9
		8	165	7

It is to be seen that in every case the percentage of neck rot in the immature bulbs was practically double that in the mature bulbs handled under identical conditions. The amount of neck tissue allowed to remain on the bulbs did not materially affect the extent of neck-rot infection, although the relative amounts of succulent tissue exposed is probably the important factor involved. In confirmation of this the writer has data for 1915 which show the Red Globe crop of onions in the Racine district was reduced from 20 to 50 per cent in storage by mycelial neck rot. The growing season of that year was unusually cool and moist, and as a result the onions matured some four weeks later than usual and in most cases were harvested before the neck tissue was thoroughly dried out. Likewise in 1924, an unusually rainy season, the losses in all varieties of onion in this and in the Chicago district due to mycelial neck rot were as great as those in 1915. As usual, the disease was most severe on the white varieties.

It is unfortunate that the writer was unable to make parallel tests with a white variety. Later studies [5] have shown that the basic principle in resistance to neck rot among colored varieties of onion is due to a host toxin which is affiliated with the scale pigments and becomes functional as the outer scale tissue dies and permits its diffusion into drops of moisture on the exterior. The exposure of the succulent neck tissue of the immature bulbs often allows the neck-rot organism to gain entrance without contact with the toxin of the dead pigmented scale tissue, whereas, in the case of the mature neck, the parasite is more liable to come in contact with this toxin. With the white varieties the effect of this toxin does not enter in. Although the mature neck tissue is undoubtedly less favorable to infection than the immature neck tissue, the difference is not due, as it is in part in the colored varieties, to the presence or absence of the inhibitory toxin. More experimental work on this point is contemplated.

EFFECT OF REMOVAL OF TOPS UPON INFECTION

In order to estimate the bearing of the removal of tops at harvest time upon infection with the mycelial neck-rot fungus, some artificial inoculations were first performed. Bulbs of White Portugal, Yellow Globe, and Red Globe varieties, the tops of which had fully matured, were harvested at Madison, Wis., on September 3 from soil which had not grown onions for at least six years. The tops were left intact on a portion of each variety, while from a similar amount of each variety the tops were cut close to the bulb so as to expose the succulent tissue. The bulbs were then sprayed with a spore suspension of the fungus. A number of each variety, topped and untopped, were sprayed with distilled water as controls. All were then placed in a moderately humid chamber at about 18° C. After 19 days the bulbs were examined, the percentage of neck rot recorded being given in Table II.

In the experiment a much larger percentage of infection occurred in topped than in the untopped bulbs, and there is little doubt that the removal of tops offers greater opportunity for infection. It is to be noted, however, that the fungus was not entirely excluded by leaving the tops intact, and the question naturally arises whether under more favorable environment even more infection might not have occurred in the untopped bulbs. The function of the inhibitive toxin already cited [5] in the pigmented neck tissue also enters into consideration when the amount of infection in colored and uncolored untopped bulbs is noted. It is significant in this connection that the highest percentage of infection in untopped bulbs occurred in the white variety. This difference becomes more striking in the field experiments to be discussed presently.

TABLE II.—*Development of mycelial neck rot in topped and untopped bulbs of White Portugal, Yellow Globe, and Red Globe onions 19 days after artificial inoculation*

Variety	Method of handling tops	Inoculated		Control	
		Total number of bulbs	Per cent neck rot	Total number of bulbs	Per cent neck rot
White Globe	Topped	21	90	20	0
Do	Untopped	19	16	20	0
Yellow Globe	Topped	10	80	9	11
Do	Untopped	10	10	10	0
Red Globe	Topped	10	80	10	0
Do	Untopped	10	0	10	0

During the years 1917, 1918, and 1920 some comparative field studies were made. Since the Red Globe onion is the type grown almost exclusively in the Racine section, observations during the first two years were limited to that variety. In 1920 special plantings of Yellow Globe and White Globe were made alongside Red Globe for the purpose of this study. In each season when the crop was mature several bushels were taken from a small portion of the field and the bulbs were impartially divided into two lots, those of one being placed in crates with the tops intact and those of the other with the tops clipped. The crates were stacked in the field to cure for several weeks and were then removed to storage. Final notes were taken two to three months later.

The data accumulated during three seasons is given in Table III. There

[5] WALKER, J. C., and LINDEGREN, C. C. FURTHER STUDIES ON THE RELATION OF ONION SCALE PIGMENTATION TO DISEASE RESISTANCE. Jour. Agr. Research 29: 507–514. 1924.

was always a difference in favor of the untopped lots with the colored varieties. These are in the main so resistant to neck rot, however, that the importance of the difference is slight even though consistent. A striking comparison between the resistance of colored and white varieties in 1920 is given. All lots were rained upon twice during curing, and environing conditions were thus quite favorable for the disease. In spite of this, the untopped colored varieties remained free from disease, while the topped bulbs showed slight amounts. With the White Globe variety heavy infection occurred and the difference between the topped and untopped lots was negligible. It appears, therefore, that when favorable conditions for infection prevail, this variety may be expected to succumb regardless of the method of handling tops at harvest. On the other hand, some advantage may be expected with the colored varieties in an average season if the tops are left intact during storage.

TABLE III.—*The effect of removal of onion tops at harvest time upon the occurrence of mycelial neck rot; experiments conducted at Racine, Wis., 1917–1920*

Year and variety	Topped or untopped	Crate No.	Total number of bulbs included	Percentage of bulbs affected by neck rot at end of storage period
				Per cent
1917 Red Globe	Untopped	1	271	1
		2	234	(a)
		3	207	(a)
		4	219	(a)
		5	233	(a)
	Topped	6	292	5
		7	295	5
		8	315	6
		9	326	4
1918 Red Globe	Untopped	1	136	4
		2	156	2
	Topped	3	127	9
		4	165	7
1920				
Red Globe	Untopped	1	365	0
	Topped	2	340	(a)
Yellow Globe	Untopped	3	297	0
	Topped	4	323	6
White Globe	Untopped	5	304	52
	Topped	6	338	56

[a] Less than 1 per cent.

INFLUENCE OF ARTIFICIAL CURING UPON INFECTION

The evidence already presented has pointed to the importance of the condition of the neck tissue of the onion bulb when exposed to the fungus in determining the amount of neck-rot infection. These data, combined with general field observations, suggested the possibility that rapid artificial curing of bulbs immediately after harvest might accomplish two things favorable to the control of neck rot. It would desiccate the neck tissues immediately so as to make them unfavorable for infection; and it would permit earlier removal to storage and thus facilitate better protection of the crop from inclement weather following harvest.

Curing experiments were conducted in 1917 and the results have already been briefly reported.[6] Further experiments on a large scale were conducted in 1918, but conditions were so unfavorable for neck rot in that year that practically no disease developed even in the controls. Further experiments were run in 1923 with favorable results. It is therefore considered advisable to report the experiments of 1917 and 1923 in some detail.

A very simple arrangement for drying was used in the 1917 experiments. A small room equipped with a coal heater was arranged so that the temperature could be raised to 100 or 110° F. Air circulation was provided by raising a double window about 4 inches from the bottom and lowering it an equal amount from the top. The onions were placed in shallow layers in standard slatted crates used for storage of onion sets. These were so placed as to secure the maximum benefit from the circulating warmed air. It was not possible to keep a uniform temperature by this means. A continuous record of temperatures was kept, and in the tabulated data the total number of hours above 90° F. for any given period is stated. In 1923 a specially constructed chamber was used, through which warmed air was forced by means of an electric blower. By the latter process the same amount of drying was secured in much less time.

EXPERIMENTS WITH ONION SETS.—Two lots of White Portugal onion sets were treated in 1917. The first of these (Lot I) was grown upon soil

[6] WALKER, J. C. CONTROL OF NECK ROT AND ANTHRACNOSE OF ONION SETS. (Abstract) Phytopathology 8 : 70. 1918.

which in the previous year had produced onions for the first time. The sets were harvested on August 16 and placed in the standard shallow crates and the latter were stacked in the field to cure in the usual manner. During the next two weeks clear bright weather prevailed with no rain, so that conditions for natural curing were as good as ever occurs under Wisconsin conditions. On August 30, 12 crates of these onions were removed to the warehouse and they were divided into two lots of 6 crates each. No sign of neck rot was noted at this time, although later observations indicate that infection had already occurred. Five crates of one lot were placed in the drier on September 3, and one crate was reserved as control.

A second lot of White Portugal onion sets was secured from an adjoining field where onions had been grown for the first time. These were harvested on August 30 and were removed to the warehouse at once without the usual field curing. There was no evidence of neck rot at this time. One crate was given no further treatment, but was so placed as to afford free air circulation and protection from rain. One crate was placed in the drier on September 3, and kept there for seven days. The remaining two crates were kept damp for one week. One of these was given no further treatment; the other was placed in the drier for seven days.

After treatment, the onions were stored in a standard onion warehouse

TABLE IV.—*The effect of artificial drying upon the development of mycelial neck rot in White Portugal onion sets, 1917*

Lot No.	History previous to experiment	Nature of treatment	Crate No.	Number of days in drier	Hours above 90° F.	Condition at end of storage period: Bulbs showing neck rot	Condition at end of storage period: Shrinkage in weight
						Per cent	*Per cent*
I	Grown on soil which had produced onions previous year for first time. Harvested Aug. 16; cured in standard crates in field for two weeks of dry, clear, and thus very favorable weather. Removed to warehouse Aug. 30.	All but crate No. 1 placed in drier Sept. 3.	1	0	(1)	9	18
			2	2	28	3	11
			3	4	47	2	11
			4	6	58	3	11
			5	8	88	1	10
			6	16	164	0.5	11
		All artificially dampened and kept damp for one week; then all but crates 7 and 8 placed in drier Sept. 10.	7	0	(1)	50	55
			8	0	(2)	27	45
			9	2	21	7	19
			10	6	57	9	17
			11	9	92	9	29
			12	14	117	10	17
II	Grown on soil new to onions. Harvested Aug. 30 and removed to warehouse at once.	No further treatment	13	0	(1)	44	49
		In drier on Sept. 3	14	7	73	7	19
		Kept damp one week: In drier Sept. 10	15	7	65	10	26
		Kept damp one week: No further treatment	16	0	--------	92	97

[1] Untreated. [2] Dried in sun.

One crate was then removed from the drier to the warehouse at two-day intervals. In this and subsequent trials the outer scales of the bulbs appeared to be thoroughly dry after exposure of two days, but the treatment was extended much longer in order to determine any possible benefit of longer exposure.

In order to increase possibilities of infection and to simulate extreme weather conditions, the remaining six crates of sets were dampened and kept damp for one week. One crate was then set aside without further treatment. Another was dried in the sun for several days according to the usual commercial method. The remaining four crates were placed in the drier and one crate removed after 2, 6, 9, and 14 days, respectively.

at Racine. On January 4 following, final notes were taken. The percentage of neck rot was estimated by examining several hundred sets in each crate. Each crate was then given the usual commercial sorting in which all excess chaff and decayed bulbs were removed. The weight of marketable sets was then determined and the shrinkage by weight for each crate was calculated. The results are summarized in Table IV.

In spite of the good weather conditions which prevailed, 9 per cent of the bulbs in Lot I and 44 per cent of the bulbs in Lot II decayed in the untreated crates. Where either lot was exposed to damp conditions for a short period the amount of infection was in each case greatly increased. In all cases the artificial drying showed bene-

ficial results. Even in the best portion of Lot I (crates Nos. 1 to 6), where only 9 per cent neck rot appeared in the control, the amount of infection was reduced two-thirds or more. In the dampened portions of Lot I (crates Nos. 7 to 12) drying by natural agencies (crate No. 8) reduced infection from 50 to 27 per cent, but artificial curing reduced the disease to the almost negligible amount of 7 to 10 per cent. Thus, by the latter treatment, damage due to the exposure to a week's damp weather was prevented.

In Lot II, where the amount of disease reached much higher proportions, the damage from neck rot was reduced to a negligible quantity by means of artificial drying. There was almost a total loss when this lot was exposed to damp conditions (crate No. 16), but immediate artificial drying again prevented such damage (crate No. 15). Representative amounts of healthy onions at the end of the storage period from crates 13, 14, and 16 are shown in Plate 2.

In comparing the amount of neck rot in crates exposed to drying for various intervals it is noteworthy that in each case (crate No. 2 and crate No. 9) practically the maximum reduction of disease was reached within two days' exposure. By forced circulation of warm dry air, the length of treatment can undoubtedly be reduced much more.

EXPERIMENTS WITH LARGE BULBS

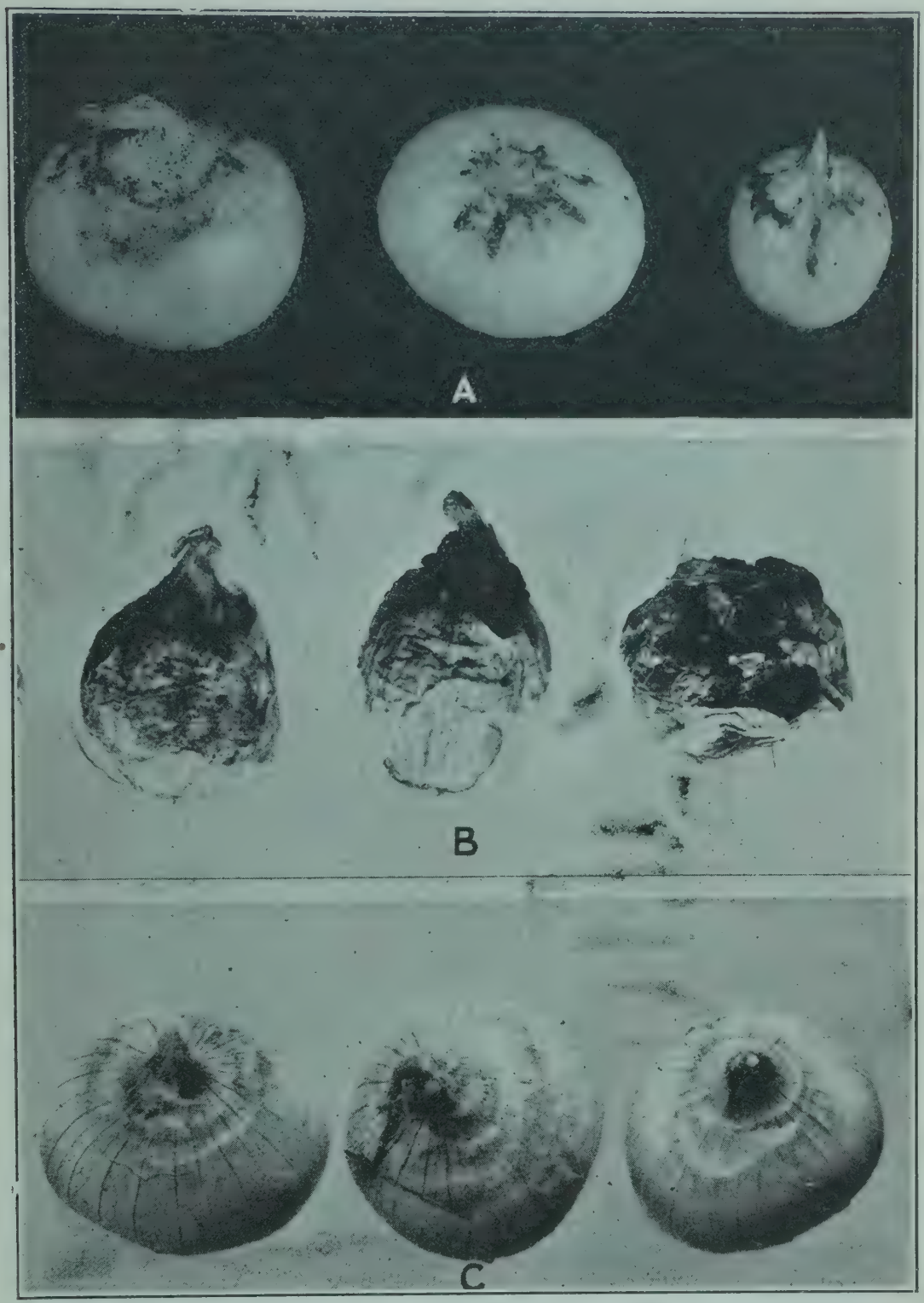
Two experiments were conducted with large bulbs, Lot III in 1917 and Lot IV in 1923. Lot III consisted of the oversize bulbs sorted out at harvest time from the sets which comprised Lots I and II. A few of these bulbs showed signs of incipient decay before they were placed in the drier. Evidence of such early infection is shown in Plate 1, A. The exposure to warm, dry air resulted in practically all cases in thorough dehydration of the diseased tissue in such bulbs. Observation throughout the storage period showed that in practically all cases this treatment checked any further advance of the fungus. The results with dried and untreated portions of Lot III are given in Table V. Almost complete elimination of loss from neck rot was attained. Typical bulbs from the dried lot in which desiccation of the slightly decayed neck occurred are illustrated as they occurred at the end of the storage period in Plate 1, B. Completely decayed bulbs, typical of the untreated lot, are shown in Plate 1, C.

Lot IV consisted of large White Globe onions grown on old onion soil at Racine, Wis., in 1923. They were harvested about September 10 and stacked in the field in slatted bushel crates for about three weeks before being placed in the drier on October 2. At the latter date there was evidence of neck rot in its early stages in a goodly percentage of the bulbs. In these the decayed tissue dried down in the same manner noted for Lot III, and in the majority of cases this resulted in a complete check of the fungus. The length of exposure and the temperature during the period of exposure with the final notes taken on December 14, after about two-months' storage are given in Table V. Although the neck tissue of large bulbs is naturally more difficult to dry out satisfactorily, it was possible to accomplish this with forced circulation of air for two or three days. The air current was maintained at room temperature, except for three to six hour intervals, during which the temperature was raised to 40° C. or 44° C.

The amount of actual decay due to neck rot was in each case reduced to an almost negligible quantity compared with that which developed in the control. The percentage of bulbs showing complete check of incipient neck-rot infection is also significant. Of course, more prompt drying of bulbs after harvest would have eliminated the incipient decay. It is valuable, however, to know that with proper treatment the disease can be checked even after the signs of the disease have become evident.

CONCLUSIONS

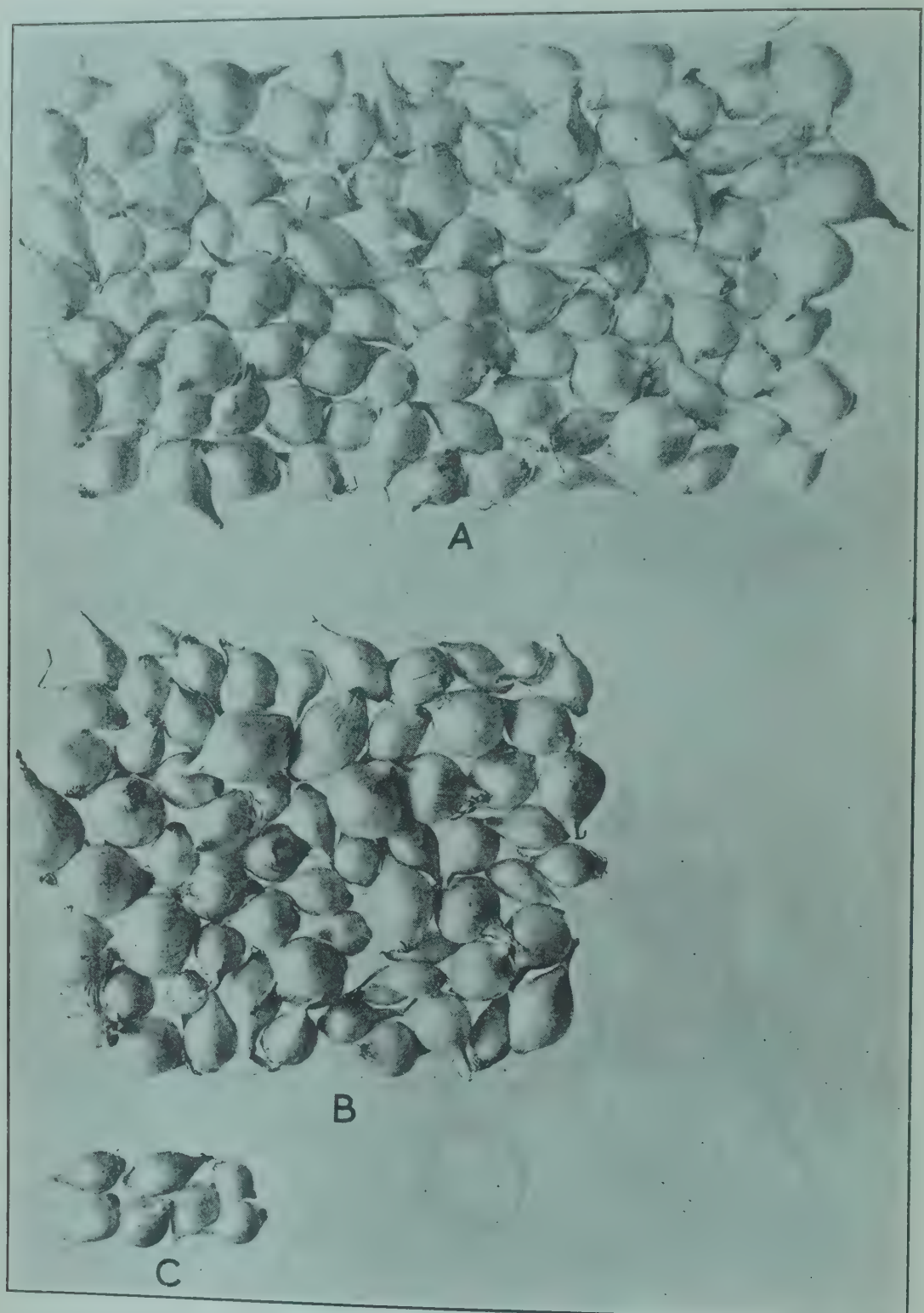
The data presented, together with field observations over a period of years, show that in the Middle West and probably in all of the Northern States several factors enter into the determination of the amount of infection by the mycelial neck rot disease. Weather conditions preceding and during harvesting and curing of crop are undoubtedly influential, and more study of their exact relation would be desirable. It is apparent that the state of maturity of the neck tissue of the bulbs at harvest is an important factor. Removal of tops may increase infection, but it is evident that this operation may under some conditions have little influence in the case of white varieties. The grower is unable by any ordinary means to so handle the crop as to prevent neck-rot infection, and this is especially the case with the white varieties, which are much more susceptible than colored varieties.



A.—Early stage in the development of the disease. Note shrinkage of the scale progressing from the neck downward, followed by the development of the gray mycelial felt on the decayed tissue

B.—Advanced stage of decay after several months in storage. Desiccation of the rotted tissue leaves dry, shriveled mummies. Note the bulb at the left is covered with conidiophores and conidia of the fungus, while the more common symptom shown in the other two bulbs consists of the large sclerotia without sporulation. Samples taken from the untreated portion of Lot III (see Table V and the text)

C.—Typical bulbs from the treated portion of Lot III. The disease was apparent at the time of drying, but the treatment resulted in desiccation of the decayed tissue at the neck and in the check of the fungus. These bulbs were photographed at the end of the storage period



The effect of artificial curing on the development of mycelial neck rot. Representative amounts of healthy onions remaining at the end of the storage period from equivalent portions of white sets taken from a common source and handled as follows:

A.—Harvested August 30, 1917, placed in drying room for 7 days beginning September 3; 7 per cent neck rot developed. (See crate 14, Table IV)

B.—Harvested August 30, protected from rain and allowed to cure naturally without any artificial treatment; 44 per cent neck rot developed. (See crate 13, Table IV)

C.—Harvested August 30, exposed to damp conditions for one week then allowed to cure without any artificial treatment; 92 per cent neck rot developed. (See crate 16, Table IV)

TABLE V.—*The effect of artificial drying upon the development of mycelial neck rot in large bulbs of White Portugal and White Globe varieties, 1917 and 1923*

Lot No. and year	History of bulbs previous to experiment	Subsequent treatment	Crate No.	Length of drying period	Total number of bulbs	Condition at end of storage period	
						Bulbs decayed with neck rot	Bulbs showing incipient neck rot completely checked
						Per cent	*Per cent*
III 1917.	White Portugal "overruns" from Lots I and II (see Table IV), 1¼ to 1¾ inches in diameter.	Untreated	17		304	36	
		In drier 7 days (81 hours above 90° F.)	18		301	.6	
IV 1923.	White Globe large onions, grown on old onion soil; harvested about Sept. 10, cured in bushel crates about three weeks, then placed in drier Oct. 2.	Untreated	19		437	44	0
		In drier (with forced circulation of air).	20	2½ days at room temperature and 6 hours at 40° C.	1,093	9	19
			21	3 days at room temperature, 3 hours at 44° C.	630	5	37
			22	do	515	7	21

The experimental evidence shows that mycelial neck rot may be largely prevented by thorough desiccation of the neck tissue of the bulbs within the first two to three weeks after harvest. It is shown that this may be accomplished by exposing the bulbs to higher temperature, by exposing them to forced air currents, or by a combination of the two methods. The principle of this measure of control seems to be sound. There remains to be worked out details whereby artificial curing can be applied on a large commercial scale with satisfactory rapidity and at a reasonable cost.

It is desirable that this investigation be extended to include the gray-mold neck rot *Botrytis allii*. Although this disease is ordinarily of minor consequence in the Illinois and Wisconsin sections, it is nevertheless widespread in its distribution and often causes serious losses in other sections. The life history of the causal organism is so similar to that of mycelial neck rot that the same methods of handling would probably lead to effective control.

SUMMARY

(1) The mycelial neck rot of onion, caused by an undescribed species of Botrytis, herein referred to as *Botrytis sp. 110*, is a common cause of heavy storage losses of onion, especially of the white varieties. This paper is concerned with the relation of certain methods of handling the onion crop at harvest and in storage to infection and to the control of the disease.

(2) When removal of onion tops at harvest is practiced, the state of maturity of the plant has a direct bearing upon the amount of infection. In the experiments with the Red Globe variety the percentage of bulbs infected was doubled in the case of lots in which the neck tissue was still succulent at time of harvest as compared with those in which the tops had matured properly.

(3) In the case of colored varieties the removal of tops as compared with allowing them to remain intact after harvest usually resulted in greater infection. In the one experiment with White Globe variety there was little difference to be noted.

(4) Artificial curing of onion bulbs sufficient to cause desiccation of the neck tissue within two or three weeks after harvest has resulted in all cases in a material reduction in the amount of disease. This treatment promises to become an effective control measure, provided it can be adapted to commercial use at a reasonable cost.

RELATIVE SUSCEPTIBILITY OF SELECTIONS FROM A FULGHUM-SWEDISH SELECT CROSS TO THE SMUTS OF OATS [1]

By GEORGE M. REED,[2] *formerly Pathologist in charge of Smut Investigations*, and T. R. STANTON, *Agronomist in charge of Oat Investigations, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Little is known concerning the reaction to the smuts of oats shown by selections from crosses between very resistant and very susceptible varieties. Definite information on the nature of infection, as well as on the mode of inheritance of smut resistance or smut susceptibility in such crosses, is essential for the breeding and development of smut immune varieties. The writers are cognizant of the fact that the data presented herein were obtained from studies of one cross only, but they seem conclusive enough to warrant publication. They are certainly an indication of the possibilities of the development, through hybridization, of strains of oats resistant to smut. It is evident that the production of economically desirable varieties of oats immune from, or highly resistant to, smut will be an important phase of varietal improvement in the future.

REVIEW OF LITERATURE

The studies of Biffen (*4*) [3] clearly demonstrated that resistance of wheat to *Puccinia glumarum* is inherited in typical Mendelian fashion. Biffen crossed the susceptible Red King with the resistant Rivet. The F_1 generation was susceptible. The F_2 progeny segregated into *susceptible* and *resistant* in an approximately three to one ratio. These results of Biffen recently have been confirmed by Armstrong (*2*).

Investigations of crosses between varieties of wheat susceptible or resistant to *Puccinia graminis* have been made by a number of workers. Hayes, Parker, and Kurtzweil (*11*) found that resistance was dominant in crosses between certain varieties of *Triticum vulgare* and *T. dicoccum*. On the other hand, susceptibility was dominant in crosses of *T. vulgare* and *T. durum*. There also was a strong linkage between rust resistance and the durum characters in the F_2 generation. Puttick (*13*), Melchers and Parker (*12*), Aamodt (*1*), Garber (*8*), Harrington and Aamodt (*9*), and Hayes and Aamodt (*10*) have contributed additional data on the general problem of the inheritance of resistance to *Puccinia graminis*, the organism causing stem rust.

Gaines (*6*) has studied the inheritance of resistance to bunt or stinking smut (*Tilletia tritici*) of wheat. He crossed the resistant Turkey and the susceptible Hybrid No. 128, and also Turkey and Florence, both of which were resistant. In the first case, all degrees of resistance in the F_2 progeny were obtained. In the second cross, certain F_2 progenies proved to be more susceptible than either parent.

Gaines, (*7*) in a second paper on the inheritance of resistance to bunt, states that this character in wheat is not a simple Mendelian unit character but, if Mendelian, is composed of multiple factors. He further states that different wheat varieties possess different kinds of resistance, and that linkage between resistance and morphological characters is not sufficient to hinder the selection of resistant strains of any type morphologically desirable.

Wakabayashi (*18*) has studied the behavior of a cross between Red Rustproof and Black Tartar oats in reference to covered smut, *Ustilago levis*. The former variety is highly resistant while the latter is moderately susceptible. The F_1 and F_2 plants produced no smut. In the F_3 generation, however, 12 families out of a total of 95 were observed to contain a number of infected individuals. In no case, however, was the number very large.

[1] Received for publication June 2, 1924; issued April, 1925, as Brooklyn Botanic Garden Contribution No. 39. Data for 1919 and 1920 obtained from investigations conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Agricultural Experiment Station of the University of Idaho. Data for 1922 obtained from the Brooklyn Botanic Garden.

[2] Now curator of plant pathology at the Brooklyn Botanic Garden, Brooklyn, N. Y.

[3] Reference is made by number (italic) to "Literature cited," p. 391.

Barney (*3*) recently has reported on some studies of the inheritance of resistance to loose smut of oats in crosses between Fulghum and Black Mesdag, Swedish Select and Burt, and Turkish Rustproof and Golden Rain. The first cross involved two very resistant varieties, the second a resistant and a susceptible variety, and the third two moderately susceptible varieties. Barney suggests that resistance in the first cross depended upon three different factors, in the second upon two, and in the third upon only one. He also has shown that, in the F_2 generation, highly resistant as well as highly susceptible families which breed true can be isolated.

The data here presented were obtained from a cross between Fulghum and Swedish Select. Reed (*14*) and Reed, Griffiths, and Briggs (*15*), who have made extensive studies on the resistance of oat varieties to loose smut, *Ustilago avenae* (Pers.) Jens., and covered smut, *U. levis* (K. and S.) Magn., found that various strains of Fulghum were highly resistant, while strains of Swedish Select were moderately susceptible.

RELATIVE SUSCEPTIBILITY

All of the tested strains of Fulghum and Swedish Select have been grown at one or more of the following stations: Columbia, Mo.; Ames, Iowa; Aberdeen, Idaho; Manhattan, Kans.; Pullman, Wash.; and Brooklyn, N. Y. All have been grown two or more years in the series inoculated with *Ustilago avenae*. The same is true in the series inoculated with *U. levis*, except that two strains were grown in only one year at one place.

FULGHUM

Four different strains of Fulghum oats, including two of Kanota, have been grown. These strains, at every station in every year, have consistently shown a high degree of resistance to both *Ustilago avenae* and *U. levis*. The highest percentage of infection (2.4 per cent) by *U. avenae* was obtained at Aberdeen in 1920, and the highest percentage of infection (3.2 per cent) by *U. levis* occurred at Manhattan in 1920.

At all the different stations 10,803 plants were grown in the *Ustilago avenae* series. These showed 0.6 per cent of infection. From seed inoculated with *U. levis* 10,200 plants were grown. These showed 0.4 per cent of infection.

SWEDISH SELECT

Six different strains of Swedish Select oats have been grown at the several stations in order to determine their susceptibility to *Ustilago avenae* and *U. levis*.

Results from inoculation with *Ustilago avenae* show that the highest percentage of infection (42.6 per cent) was obtained in strain No. 225 at Brooklyn, N. Y. The lowest percentage of infection (1.1 per cent) occurred at Brooklyn in 1922, in strain No. 226 (C. I. No. 134)[4] and also No. 227. All of the strains at some locality have had percentages of infection above 32.3 per cent except strain No. 229 (C. I. No. 1743). Of all the strains at the different stations 2,694 plants have been grown and 19.9 per cent were infected.

Results with *Ustilago levis* show that the highest percentage of infection (45.1 per cent) was obtained in strain No. 168 at Columbia. High infections of 39.7 per cent and 40.2 per cent, respectively, were obtained in strains Nos. 226 (C. I. No. 134) and 227. The lowest percentage of infection obtained was 3.4 per cent in strains No. 226 (C. I. No. 134) and 229 (C. I. No. 1743) at Brooklyn. In the entire series, 2,172 plants were grown, and 17.6 per cent of these were infected.

Where the data are sufficient for comparison there seems to be no obvious difference in the susceptibility of these strains to the two different smuts. Infections above 40 per cent are only occasional.

PARENT VARIETIES

Fulghum and Swedish Select are important varieties of oats in the United States. They are representative of the two groups of oats to which practically all our cultivated varieties belong, namely, *Avena sativa* L. and *A. byzantina* C. Koch. Panicles and spikelets of these varieties are shown in Plate 1.

Fulghum is one of the leading varieties of so-called red oats grown in this country. As already noted, it has shown a high degree of resistance to smut. It is grown most extensively in the Southern States and in Kansas, where it has recently become popular under the name Kanota (*16*).

Swedish Select is one of our principal midseason white varieties, and is grown extensively throughout the northern United States. In the va-

[4] C. I. No. = Cereal Investigations accession number, here and throughout this report.

Panicles and spikelets of the parent varieties, Fulghum (at left) and Swedish Select (at right) (approximately two-thirds natural size)

rietal survey made by the Office of Cereal Investigations in cooperation with the former Bureau of Crop Estimates, it was estimated that 3,631,789 acres were devoted to this variety in 1919. In total acreage it is exceeded only by Silvermine and Red Rustproof.

FULGHUM

HISTORY.—Fulghum originated as a selection from the Red Rustproof variety in southeastern Georgia about 20 years ago, where it originally was grown entirely as a fall-sown variety. It has become of importance only as a spring-sown variety in recent years. A complete history of Fulghum has been recorded by Stanton (*17*).

DESCRIPTION.—Early growth semi-spreading to erect; plant very early. Culms midsized, strong, usually glabrous, 70 to 120 cm. tall. Sheaths deep green, glabrous; culm leaves midwide, margins ciliate. Peduncle midsized, straight, well exserted. Panicle equilateral, erect, small, midlong, narrow, ovate; rachis nodes 4 to 7; branches short, ascending, scabrous. Spikelets usually few, apex spikelets usually 3-flowered; kernels slender to midplump. Empty glumes 22 to 28 mm. long, 6 to 8 mm. wide, usually 8-veined, light green before maturity. Lower lemma reddish yellow (buff) 17 to 21 mm. long, glabrous, basal scar prominent to obscure; basal hairs usually present, few, short to long; awn present or absent, straight (non-twisted), 15 to 30 mm. long. Upper lemma 12 to 16 mm. long, rarely awned, usually attached to the lower, on separation the midlong, glabrous upper rachilla segment usually remaining attached to the lemma.

Fulghum is differentiated from the related Red Rustproof variety primarily by its more erect panicle, slightly less spreading habit in early growth, more slender and lighter-colored kernels with fewer awns and basal hairs. Many aberrant or so-called off-types occur in Fulghum. These vary from cultivated forms which retain their white, gray, or black kernels to the typical "false wild" oat which sheds its seeds immediately on ripening.

SWEDISH SELECT

HISTORY.—The Swedish Select variety is of Swedish origin, as the name implies, and was introduced from Russia by M. A. Carleton in 1899. The best information at hand indicates that it was introduced into Finland from Sweden and then into the St. Petersburg Province of Russia. It is said to have originated as a selection from the Ligowa oat.

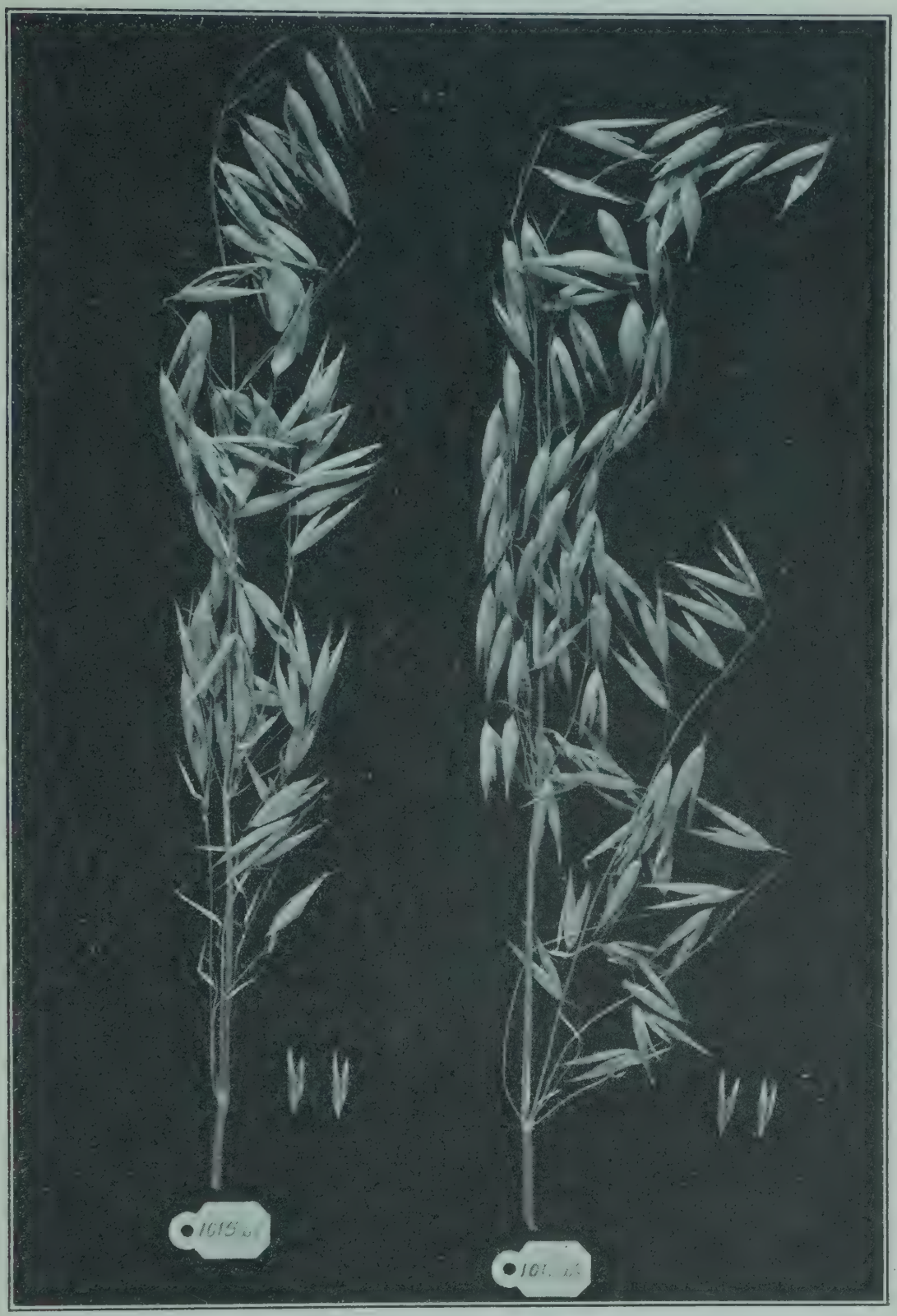
DESCRIPTION.—Early growth erect; plant midseason. Culms midsized to large, strong, usually glabrous, 80 to 130 mm. tall. Sheaths deep green, usually glabrous; culm leaves midwide, margins usually glabrous. Peduncle midsized, straight, well exserted. Panicle equilateral, usually erect, midlong, midbroad, ovate; rachis nodes 5 to 7; branches short to midlong, usually ascending, scabrous. Spikelets few to numerous; usually about 50 per cent 3-flowered; kernels very plump. Empty glumes 18 to 24 mm. long, 6 to 8 mm. wide, 8 to 9 veined, deep green and slightly glaucous before maturity. Lower lemma white, 14 to 17 mm. long, glabrous, dorsal surface distinctly depressed above the awn; basal hairs usually absent; awn usually present, very dark at base, twisted and subgeniculate to twisted and geniculate, 15 to 35 mm. long. Upper lemma 10 to 14 mm. long, awnless; rachilla segment short, glabrous.

The outstanding characters of the Swedish Select variety are its short, plump kernels distinctly depressed dorsum. The awn is very dark at the base, and usually twisted and geniculate. There also is a high percentage of 3-kerneled spikelets. These characters usually serve to separate it clearly from the otherwise similar varieties, Silvermine and Lincoln.

FULGHUM-SWEDISH SELECT CROSS

HISTORY.—This cross was made by the junior author in the greenhouse at the Arlington Experiment Farm, Rosslyn, Va., in the spring of 1916, to which the hybrid number 1015 was assigned. The two F_1 generation plants, designated as 1015a1 and 1015a2, were grown under greenhouse conditions at the same farm, from which seed was available for sowing at the Aberdeen Substation, Aberdeen, Idaho, in the spring of 1918. A portion of the seed from a number of the resulting F_2 plants was turned over to the senior author for starting the studies reported herein. Panicles and spikelets of the two F_1 plants are shown in Plate 2.

Some brief notes were recorded on the appearance of the two F_1 plants when they were approaching maturity. Both were more vigorous than either parent and in general appearance they resembled Fulghum more than Swedish Select in most characters. The two plants averaged 145 cm. in height, as compared with 110 cm. and 140 cm., respectively, for the Fulghum and Swedish Select parents. The reddish tinge of the culms, empty glumes, etc., so characteristic of the Fulghum and



Panicles and spikelets of the two F_1 plants, 1015 a1 (at left) and 1015 a2 (at right) of the Fulghum-Swedish Select cross (approximately two-thirds natural size)

other red oat varieties, was very evident in the F_1 plants. The lemmas were reddish-yellow like those of Fulghum, but in shape they were rather plump like those of Swedish Select. One plant was practically awnless, while about 25 per cent of the spikelets on the other were awned. The awns were somewhat twisted and dark at the base like those of the Swedish Select parent, but only slightly bent. It is thus seen that in respect to the presence of awns this cross behaved similarly in the F_1 to the Sixty-Day × Burt cross, reported by Fraser (*5*).

SECOND GENERATION PROGENY

Altogether, 118 F_2 plants were grown from the seed of the two F_1 plants at the Aberdeen Substation, Aberdeen, Idaho, in 1918. Samples of these plants have been preserved and an attempt has been made to classify them with regard to the degree or extent of awning, disjunction of the second floret, and color of lemma, primarily to determine if any relationship exists between these characters and smut resistance. It is fully realized that the number of F_2 plants is entirely too small for the determination of genetic ratios. Further, an extended discussion on the inheritance of these characters is not within the province of this paper.

Of the 118 plants, 25 were classed as having few or no awns (awnless), 76 as having abundant awns (fully awned), and the remaining 17 as being intermediate or commonly awned (approximately 50 per cent of the spikelets awned). These numbers do not even indicate the ratio of 1 awnless, 2 partly awned, and 1 fully awned, obtained by Fraser (*5*) from a cross between an awnless strain of Sixty-Day and Burt, the latter an exceedingly variable variety with regard to awns and other kernel characters. Provided the mode of inheritance is the same in this cross, the small F_2 population may account for this wide deviation from the expected ratio. However, the preponderance of awns in the F_2 plants probably is best explained by the fact that Swedish Select usually is a rather fully awned variety, and that frequently in Fulghum as many as 50 per cent of the spikelets are awned. As a consesequence, it could not be expected that the genetic behavior regarding awns would be the same as in a cross where one parent is awnless and the other only partly awned.

Of the 76 plants with abundant awns, about 16 showed the twisted and geniculate awn frequently occurring in the Swedish Select parent, which type of awn apparently occurs less frequently than the nontwisted straight awn of the Fulghum parent.

Classification with regard to the disjunction of the second floret or lemma of the spikelet (separation of second floret from the first) was most difficult. Those plants in which the second floret was attached rather firmly to the first, and on separation usually carried its rachilla segment with it, were classed as attached (sterilis of Fraser), while those in which the second floret disarticulated easily, the rachilla segment usually remaining on the first floret, were classed as disarticulated (sativa of Fraser). The remaining plants in which no differentiation was possible were classed as intermediate. On this basis, therefore, 25 were classed as attached, or as having the disjunction of the Fulghum, and 13 as disarticulated, or as having the disarticulation of Swedish Select, and 80 as intermediate between the two. Fraser (*5*), in his observations on the cross Sixty-Day × Burt, obtained a ratio of 1 sterilis, 2 intermediate, and 1 sativa. The numbers obtained in the present cross indicate this ratio, but do not approach it very closely.

On the basis of color of lemma, classification was even more difficult than in the case of awning and disjunction. Because of the blending of one color into the other in the same plant no very satisfactory classification could be made. The color of the lemmas varied from reddish-yellow to yellowish-white. There were really three colors present which have been described as reddish-yellow, yellow, and yellowish-white. However, differentiation between reddish-yellow and yellow, as well as between yellow and yellowish-white, was most difficult in some plants. Of the 118 plants about 32 were classed as not definitely falling into the reddish-yellow or yellow class. Of the remaining 86, there were 28 of a reddish-yellow typical of the color in Fulghum, and 58 lighter in color, and in most cases a true yellow. The dominance of the reddish-yellow color of the Fulghum over the white of Swedish Select in the F_2 generation of the cross in question, is indicated.

While this classification on the basis of certain kernel characters is not all that could be desired, it is believed worth while, however, in that it will indicate in some measure whether there is any relationship between morphological characters and smut resistance or smut susceptibility (pl. 3).

The spikelet descriptions of the 92 F_2 plants used in the smut-inoculation experiments reported herein are shown

Spikelets and florets of F_2 plants of the Fulghum-Swedish Select cross resistant and susceptible to the smuts of oats. Reading from left to right, 1015a1–12; 1015a2–9; 1015a1–33; 1015a2–31; 1015a1–45; 1015a2–37, resistant; 1015a1–52; 1015a2–38; 1015a1–54, susceptible, and 1015a2–54, resistant

TABLE 1.—*Reaction of F_3, F_4, and some F_5 progenies from the hybrid No. 1015a, Avena byzantina variety Fulghum × A. sativa variety Swedish Select, to Ustilago avenae (Pers.) Jens., and U. levis (K. & S.) Magn., with descriptions of certain spikelet characters of the F_2 parent plants* [a]

Hybrid number	Generation	Station and year	Spikelet characters in F_2 plants			Plants from seed inoculated with—					
			Awns	Rachilla segment of second floret	Lemma color	Ustilago avenae			Ustilago levis		
						Total	Infected		Total	Infected	
						Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
1015a1-1	F_3	Aberdeen, 1919	Abundant	Intermediate	Reddish-yellow	35	4	11.4			
1a	F_4	Brooklyn, 1922				40	4	10.0	48	1	2.1
2	F_3	Aberdeen, 1919	Abundant	Attached	Yellow	74	18	24.3			
2a	F_4	Brooklyn, 1922				109	2	1.8	109	10	9.2
3	F_3	Aberdeen, 1919	Common	Intermediate	Yellow	54	8	14.8			
3a	F_4	Brooklyn, 1922				101	0	0	85	2	2.4
4	F_3	Aberdeen, 1919	Common	Intermediate	Reddish-yellow	63	6	9.5			
4a	F_4	Brooklyn, 1922				61	3	4.9	76	3	4.0
5	F_3	Aberdeen, 1919	Few	Disarticulated	Yellowish-white	83	6	7.2			
5a	F_4	Brooklyn, 1922				98	2	2.0	66	3	4.5
6	F_3	Aberdeen, 1919	Abundant [b]	Attached	Yellow	65	22	33.8			
6a	F_4	Brooklyn, 1922				71	5	7.0	115	0	0
7	F_3	Aberdeen, 1919	Few	Intermediate	Yellowish-white	4	0	0			
7a	F_4	Brooklyn, 1922				57	0	0	59	2	3.4
8	F_3	Aberdeen, 1919	Common	Intermediate	Yellowish-white	10	3	30.0			
8a	F_4	Brooklyn, 1922				67	1	1.5	34	3	8.8
10	F_3	Aberdeen, 1919	Common	Intermediate	Yellow	41	6	14.6			
10a	F_4	Brooklyn, 1922				96	0	0	75	1	1.3
11	F_3	Aberdeen, 1919	Common	Intermediate	Yellowish-white	48	14	29.2			
11a	F_4	Brooklyn, 1922				126	2	1.6	94	34	36.2
12	F_3	Aberdeen, 1919	Common	Attached	Yellowish-white	54	0	0			
12a	F_4	Brooklyn, 1922				106	0	0	154	0	0
13	F_3	Aberdeen, 1919	Abundant	Intermediate	Yellowish-white	22	9	40.9			
13a	F_4	Brooklyn, 1922				44	0	0	39	1	2.6
14	F_3	Aberdeen, 1919	Abundant [b]	Intermediate	Yellowish-white	70	0	0			
15	F_3	do	Abundant	Attached	Reddish-yellow	62	10	16.1			
15a	F_4	Brooklyn, 1922				108	2	1.9	96	10	10.4
16	F_3	Aberdeen, 1919	Common	Attached	Yellow	94	12	12.8			
16a	F_4	Brooklyn, 1922				97	0	0	84	6	7.1
17	F_3	Aberdeen, 1919	Abundant	Intermediate	Yellow	48	4	8.3			
17a	F_4	Brooklyn, 1922				113	0	0	57	0	0
18	F_3	Aberdeen, 1919	Few	Attached	Yellow	20	0	0			
18a	F_4	Brooklyn, 1922				42	0	0	55	0	0
19	F_3	Aberdeen, 1919	Abundant	Attached	Reddish-yellow	73	16	21.9			
19a	F_4	Brooklyn, 1922				56	0	0	117	10	8.5

21	F_3	Aberdeen, 1919	Abundant	Attached	Reddish-yellow	64	1	1.6			
21a	F_4	Brooklyn, 1922				70	0	0	65	1	1.5
22	F_3	Aberdeen, 1919	Abundant	Intermediate	Yellowish-white	4	0	0			
22a	F_4	Brooklyn, 1922				27	0	0	41	2	4.9
23	F_3	Aberdeen, 1919	Abundant	Intermediate	Yellow	54	25	46.3			
23a	F_4	Brooklyn, 1922				69	6	8.7	79	19	24.1
24	F_3	Aberdeen, 1919	Common	Intermediate	Yellow	35	7	20.0			
24a	F_4	Brooklyn, 1922				59	1	1.7	72	9	12.5
25	F_3	Aberdeen, 1919	Few	Intermediate	Yellow	68	10	14.7			
26	F_3	do	do	do	Yellowish-white	77	4	5.2			
28	F_3	do	do	do	do	68	10	14.7			
29	F_3	do	Abundant	do	Yellow	59	5	8.5			
32	F_3	do	do	do	Yellowish-white	49	6	12.3			
33	F_3	do	Few	do	do	46	0	0			
33a1	F_4	Aberdeen, 1920				55	0	0			
33a2	F_4	do				69	0	0			
33a3	F_4	do				104	3	2.9			
33a4	F_4	do				107	0	0			
33a5	F_4	do				70	0	0			
33a6	F_4	do				102	0	0			
33a1	F_5	Brooklyn, 1922				60	0	0	50	1	2.0
33a2	F_5	do				53	0	0	53	0	0
33a3	F_5	do				126	1	.8	71	0	0
33a4	F_5	do				77	0	0	110	0	0
33a5	F_5	do				79	0	0	98	0	0
33a6	F_5	do				71	0	0	97	0	0
34	F_3	Aberdeen, 1919	Few	Intermediate	Yellowish-white	75	11	14.7			
37	F_3	do	Abundant [b]	Attached	Yellow	57	2	3.5			
37a	F_4	Brooklyn, 1922				73	0	0	79	2	2.5
38	F_3	Aberdeen, 1919	Abundant [b]	Attached	Yellow	42	1	2.4			
39	F_3	do	Abundant	do	Reddish-yellow	38	2	5.3			
40	F_3	do	Few	Intermediate	Yellow	51	3	5.9			
41	F_3	do	Abundant [b]	Attached	do	67	3	4.5			
43	F_3	do	Abundant	Intermediate	do	45	9	20.0			
44	F_3	do	Abundant [b]	do	Yellowish-white	65	6	9.2			
45	F_3	do	Abundant	do	Yellow	31	0	0			
45a1	F_4	Aberdeen, 1920				84	1	1.2			
45a2	F_4	do				52	0	0			
45a3	F_4	do				68	0	0			
45a4	F_4	do				67	0	0			
45a5	F_4	do				77	0	0			
45a6	F_4	do				65	0	0			
45a1	F_4	Brooklyn, 1922				109	0	0	86	1	1.2
45a2	F_5	do				71	0	0	99	0	0
45a3	F_5	do				88	0	0	96	0	0

[a] As used in Table I, in describing the occurrence of awns, the term "abundant" means that about 75 per cent or more of the lower florets were awned; the term "common" means that approximately from 25 to 75 per cent of the lower florets were awned, and the term "few" means that less than 25 per cent of the lower florets were awned. Under the column heading "Rachilla segment of second floret," the term "attached" means that in disjunction the rachilla segment of the second floret breaks at its base where it emerges from between the in-rolled edges of the lemma of the lower floret, and remains attached to the floret it bears. The term "disarticulated" means that the rachilla segment of the second floret disarticulates at its apex where it was attached to the floret and remains on the ventral surface of the lower floret. The term "intermediate" means that in disjunction the rachilla segment of the second floret is fractured somewhere near the middle, leaving from one-third to two-thirds of its length attached to the second floret which it bore.

[b] Awns long, twisted and geniculate, dark at base.

TABLE I.—*Reaction of F_3, F_4, and some F_5 progenies from the hybrid No. 1015a, Avena byzantina variety Fulghum × A. sativa variety Swedish Select, to Ustilago avenae (Pers.) Jens., and U. levis (K. & S.) Magn., with descriptions of certain spikelet characters of the F_2 parent plants*—Continued

Hybrid number	Generation	Station and year	Spikelet characters in F_2 plants			Plants from seed inoculated with—					
			Awns	Rachilla segment of second floret	Lemma color	Ustilago avenae			Ustilago levis		
						Total	Infected		Total	Infected	
						Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
1015a1–45a4	F_5	Brooklyn, 1921				93	0	0	97	0	0
45a5	F_5	do				83	0	0	103	0	0
45a6	F_5	do				86	0	0	105	0	0
46	F_3	Aberdeen, 1919	Abundant	Attached	Yellow	65	2	3.1			
47	F_3	do	do	Intermediate	do	54	1	1.9			
48	F_3	do	Few	Disarticulated	Yellowish-white	39	8	20.5			
50	F_3	do	Abundant	Attached	Yellow	84	10	11.9			
52	F_3	do	do	Intermediate	Yellowish-white	54	19	35.2			
52a1	F_4	Aberdeen, 1920				114	49	43.0			
52a2	F_4	do				111	28	25.2			
52a3	F_4	do				74	22	29.7			
52a4	F_4	do				115	54	47.0			
52a5	F_4	do				63	30	47.6			
52a6	F_4	do				84	45	53.6			
52a1	F_5	Brooklyn, 1922				64	10	15.6	130	39	30.0
52a2	F_5	do				57	7	12.3	72	15	20.8
52a3	F_5	do				107	1	.9	52	10	19.2
52a4	F_5	do				82	7	8.5	91	20	22.0
52a5	F_5	do				142	3	2.1	96	18	18.8
52a6	F_5	do				61	5	8.2	58	8	13.8
53	F_3	Aberdeen, 1919	Abundant	Attached	Reddish-yellow	73	9	12.3			
54	F_3	do	do[b]	do	Yellow	74	43	58.1			
54a1	F_4	Aberdeen, 1920				80	42	52.5			
54a2	F_4	do				104	53	51.0			
54a3	F_4	do				90	52	57.8			
54a4	F_4	do				92	42	45.7			
54a5	F_4	do				80	51	63.7			
54a6	F_4	do				93	73	78.5			
54a1	F_5	Brooklyn, 1922				108	12	11.1	82	24	29.3
54a2	F_5	do				86	7	8.1	72	15	20.8
54a3	F_5	do				69	6	8.7	140	43	30.7
54a4	F_5	do				74	4	5.4	65	7	10.8
54a5	F_5	do				75	7	9.3	86	12	14.0
54a6	F_5	do				69	8	11.6	84	26	31.0
55	F_3	Aberdeen, 1919	Abundant	Attached	Reddish-yellow	45	2	4.4			

56	F_3	do	do	do	Yellow	41	8	19.5				
57	F_3	do	Few	Intermediate	do	38	10	26.3				
59	F_3	do	Abundant	do	do	69	44	63.8				
60	F_3	do	do	Attached	Reddish-yellow	55	21	38.2				
60a1	F_4	Aberdeen, 1920				145	63	43.4				
60a2	F_4	do				121	50	41.3				
60a3	F_4	do				118	54	45.8				
60a4	F_4	do				91	37	40.7				
60a5	F_4	do				114	47	41.2				
60a6	F_4	do				130	45	34.6				
60a1	F_5	Brooklyn, 1922				56	2	3.6	96	11	11.5	
60a2	F_5	do				95	4	4.2	91	24	26.4	
60a3	F_5	do				68	3	4.4	83	12	14.5	
60a4	F_5	do				117	3	2.6	114	18	15.8	
60a5	F_5	do				144	9	6.3	120	16	13.3	
60a6	F_5	do				117	4	3.4	86	9	10.5	
1015a2-1	F_3	Aberdeen, 1919	Abundant	Intermediate	Yellow	54	26	48.1				
2	F_3	do	Few	Attached	Reddish-yellow	40	15	37.5				
3	F_3	do	do	do	Yellow	27	14	51.9				
5	F_3	do	Abundant	Disarticulated	do	72	19	26.4				
7	F_3	do	do	Intermediate	do	73	13	17.8				
7a	F_4	Brooklyn, 1922				99	0	0	91	2	2.2	
9	F_3	Aberdeen, 1919	Abundant	Intermediate	Reddish-yellow	45	0	0				
9a1	F_4	Aberdeen, 1920				60	0	0				
9a2	F_4	do				63	2	3.2				
9a3	F_4	do				45	1	2.2				
9a4	F_4	do				67	0	0				
9a5	F_4	do				95	4	4.2				
9a6	F_4	do				78	0	0				
9a1	F_5	Brooklyn, 1922				151	0	0	142	0	0	
9a2	F_5	do				75	0	0	110	0	0	
9a3	F_5	do				67	0	0	162	0	0	
9a4	F_5	do				174	0	0	100	0	0	
9a5	F_5	do				94	0	0	98	1	1.0	
9a6	F_5	do				49	0	0	106	0	0	
11	F_3	Aberdeen, 1919	Common	Attached	Yellow	64	45	70.3				
12	F_3	do	Abundant[b]	Intermediate	do	45	5	11.1				
13	F_3	do	Few	do	do	58	8	13.8				
16	F_3	do	Common	do	do	73	9	12.3				
17	F_3	do	Abundant	Attached	do	47	27	57.4				
18	F_3	do	do	Intermediate	do	63	16	25.4				
18a	F_4	Brooklyn, 1922				74	11	14.9	59	8	13.6	
19	F_3	Aberdeen, 1919	Abundant	Intermediate	Yellow	50	1	2.0				
20	F_3	do	do	do	do	43	13	30.2				
21	F_3	do	Common	do	Reddish-yellow	61	23	37.7				
22	F_3	do	Abundant	do	do	68	6	8.8				
23	F_3	do	Common	do	Yellow	49	2	4.1				
23a	F_4	Brooklyn, 1922				40	0	0	85	18	21.2	
24	F_3	Aberdeen, 1919	Common	Intermediate	Reddish-yellow	70	19	27.1				
25	F_3	do	Abundant	do	Yellow	65	19	29.2				
25a	F_4	Brooklyn, 1922				121	5	4.1	81	4	4.9	

[b] Awns long, twisted and geniculate, dark at base.

TABLE I.—*Reaction of F_3, F_4, and some F_5 progenies from the hybrid No. 1015a, Avena byzantina variety Fulghum × A. sativa variety Swedish Select, to Ustilago avenae (Pers.) Jens., and U. levis (K. & S.) Magn., with descriptions of certain spikelet characters of the F_2 parent plants*—Continued

Hybrid number	Generation	Station and year	Spikelet characters in F_2 plants			Plants from seed inoculated with—					
			Awns	Rachilla segment of second floret	Lemma color	Ustilago avenae			Ustilago levis		
						Total	Infected		Total	Infected	
						Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
1015a2-29	F_3	Aberdeen, 1919	Abundant	Disarticulated	Yellowish-white	46	5	10.9			
30	F_3	do	do	do	do	45	22	48.9			
31	F_3	do	do	Intermediate	Yellow	49	0	0			
31a1	F_3	Aberdeen, 1920				72	0	0			
31a2	F_4	do				90	2	2.2			
31a3	F_4	do				105	0	0			
31a4	F_4	do				65	3	4.6			
31a5	F_4	do				79	3	3.8			
31a6	F_4	do				82	1	1.2			
31a1	F_5	Brooklyn, 1922				95	0	0	89	0	0
31a2	F_5	do				86	0	0	83	0	0
31a3	F_5	do				80	0	0	117	0	0
31a4	F_5	do				58	0	0	67	0	0
31a5	F_5	do				117	0	0	92	1	1.1
31a6	F_5	do				87	0	0	104	0	0
32	F_3	Aberdeen, 1919	Abundant	Intermediate	Reddish-yellow	56	8	14.3			
34	F_3	do	do	do	Yellow	46	5	10.9			
35	F_3	do	Abundant[b]	do	Reddish-yellow	46	1	2.2			
35a	F_4	Brooklyn, 1922				69	0	0	62	1	1.6
37	F_3	Aberdeen, 1919	Abundant	Intermediate	Yellowish-white	58	0	0			
37a1	F_4	Aberdeen, 1920				110	0	0			
37a2	F_4	do				97	2	2.1			
37a3	F_4	do				81	1	1.2			
37a4	F_4	do				57	0	0			
37a5	F_4	do				87	7	8.0			
37a6	F_4	do				99	6	6.1			
37a1	F_5	Brooklyn, 1922				133	0	0	94	0	0
37a2	F_5	do				110	0	0	99	0	0
37a3	F_5	do				90	0	0	104	0	0
37a4	F_5	do				87	0	0	133	0	0
37a5	F_5	do				72	0	0	84	0	0
37a6	F_5	do				121	0	0	141	0	0
38	F_3	Aberdeen, 1919	Few	Intermediate	Yellowish-white	31	0	0			
38a1	F_4	Aberdeen, 1920				50	3	6.0			
38a2	F_4	do				56	0	0			

38a3	F_4	do				82	11	13.4			
38a4	F_4	do				91	17	18.7			
38a5	F_4	do				51.	0	0			
38a6	F_4	do				59	2	3.4			
38a1	F_5	Brooklyn, 1922				125	1	0.8	61	0	0
38a2	F_5	do				129	0	0	44	0	0
38a3	F_5	do				100	1	1.0	115	3	2.6
38a4	F_5	do				74	0	0	78	5	6.4
38a5	F_5	do				63	0	0	62	1	1.6
38a6	F_5	do				86	0	0	71	3	4.2
39	F_3	Aberdeen, 1919	Abundant	Intermediate	Reddish-yellow	54	23	42.6			
40	F_3	do	do	do	Yellowish-white	70	1	1.4			
41	F_3	do	Abundant [b]	do	Yellow	54	8	14.8			
45	F_3	do	Abundant	do	do	43	2	4.7			
46	F_3	do	do	do	Yellowish-white	85	6	7.1			
46a	F_4	Brooklyn, 1922				101	3	3.0	51	4	7.8
47	F_3	Aberdeen, 1919	Few	Disarticulated	Yellowish-white	56	4	7.1			
48	F_3	do	Abundant [b]	Intermediate	Yellow	70	24	34.3			
48a	F_4	Brooklyn, 1922				64	0	0	64	5	7.8
49	F_3	Aberdeen, 1919	Abundant [b]	Intermediate	Yellow	55	12	21.8			
49a	F_4	Brooklyn, 1922				86	1	1.2	94	9	9.6
50	F_3	Aberdeen, 1919	Common	Disarticulated	Yellow	36	12	33.3			
50a	F_4	Brooklyn, 1922				86	3	3.5	70	5	7.1
51	F_3	Aberdeen, 1919	Few	Intermediate	Yellow	60	3	5.0			
52	F_3	do	Abundant	Disarticulated	Yellowish-white	53	18	34.0			
52a	F_4	Brooklyn, 1922				85	7	8.2	100	17	17.0
53	F_3	Aberdeen, 1919	Abundant	Intermediate	Yellow	36	19	52.8			
53a	F_4	Brooklyn, 1922				84	4	4.8	84	7	8.3
54	F_3	Aberdeen, 1919	Abundant	Intermediate	Yellow	54	0	0			
54a1	F_4	Aberdeen, 1920				77	0	0			
54a2	F_4	do				75	1	1.3			
54a3	F_4	do				60	1	1.7			
54a4	F_4	do				70	2	2.9			
54a5	F_4	do				89	0	0			
54a6	F_4	do				53	1	1.9			
54a1	F_5	Brooklyn, 1922				103	0	0	78	0	0
54a2	F_5	do				149	0	0	103	0	0
54a3	F_5	do				128	0	0	129	0	0
54a4	F_5	do				59	0	0	79	0	0
54a5	F_5	do				134	0	0	74	0	0
54a6	F_5	do				90	0	0	71	1	1.4
56	F_3	Aberdeen, 1919	Few	Intermediate	Yellow	21	7	33.3			
57	F_3	do	Abundant	Disarticulated	Yellowish-white	65	33	50.8			
57a	F_4	Brooklyn, 1922				66	1	1.5	97	9	9.3
58	F_3	Aberdeen, 1919	Abundant	Intermediate	Yellowish-white	55	14	25.5			
58a	F_4	Brooklyn, 1922				135	3	2.2	75	18	24.0

[b] Awns long, twisted and geniculate, dark at base.

in Table I in connection with the presentation of the data on the relative smut resistance of their progeny.

EXPERIMENTAL DATA

The first studies on the resistance of the progeny of hybrids No. 1015a1 and No. 1015a2 to *Ustilago avenae* were conducted at the Aberdeen Substation, Aberdeen, Idaho, in 1919. The seed from 49 and 43 F_2 plants, respectively, was inoculated and the F_3 plants were examined for their behavior toward the loose smut.

In 1920, the seed from individually selected F_3 plants was inoculated with *Ustilago avenae* and again grown at Aberdeen. The selected plants came from rows which showed either a very high degree of resistance or of susceptibility in the previous year.

In 1922, F_4 plants were grown from seed harvested from the F_3 rows of the 1919 sowings. However, no attempt was made to grow plants from a single F_3 parent. In 1922, also, F_5 plants from the F_4 generation of 1920 were grown. Separate lots of seed were inoculated with *Ustilago avenae* and *U. levis*.

The methods employed have been fully described in the publications of Reed (*14*) and Reed, Griffiths, and Briggs (*15*). An adequate quantity of seed for sowing a rod row was placed in a small envelope and sufficient smut spores to thoroughly dust the seed were added.

In Table I, the data obtained on the reaction of selections from the hybrid Fulghum × Swedish Select to *Ustilago avenae* and *U. levis* are presented.

DISCUSSION OF RESULTS

Reference to Table I shows that very great variation exists in the reaction of the different selections to the loose and covered smuts of oats. The percentage of infection ranges from zero to as high as 79 per cent. For the purpose of studying the relative resistance or susceptibility of each selection as indicated by its reaction to the smuts, some method of comparison is necessary. Accordingly, on the basis of the 1919 results, the following classes arbitrarily have been adopted and a certain range in percentage of infection assigned to each. The classes and infection ranges are as follows:

	Per cent
1. Very resistant	0 to 5
2. Moderately resistant	5 to 20
3. Moderately susceptible	20 to 40
4. Very susceptible	40+

On the basis of the data obtained for Fulghum and Swedish Select, the parents of the hybrids, the former would be placed in group 1 and the latter in group 2 or 3.

SMUT INFECTION AT ABERDEEN, IDAHO, IN 1919

As noted in Table I, 92 F_3 families, the seed of which had been inoculated with *Ustilago avenae*, were grown. Of these families, 25 fall into class 1, 33 families fall into class 2, and 22 families into class 3. The remaining 12 families proved to be very susceptible and fall into class 4.

The progeny of the 2 F_1 plants in general has responded in a similar fashion. A few more families of hybrid No. 1015a1 are found in the very resistant class, while on the other hand, the very susceptible class is represented by an excess of families from hybrid No. 1015a2.

SMUT INFECTION AT ABERDEEN, IDAHO, IN 1920

In 1920, the seed from six different individually selected F_3 plants from each of several families was inoculated and sown.

Selections were made from two resistant No. 1015a1 F_3 families (Nos. 33 and 45), and from five resistant No. 1015a2 F_3 families (Nos. 9, 31, 37, 38, and 54). All of these seven families had shown negative results in 1919. In 1920, the percentages of infection in all the selections from families Nos. 33 and 45 from No. 1015a1, and of Nos. 9, 31, and 54 from No. 1015a2, were either zero or very small. Two of the selections from family No. 37 of No. 1015a2 showed 8 per cent and 6.1 per cent infection, respectively. Two selections from family No. 38 of No. 1015a2 showed rather high infections, namely, 13.4 and 18.7 per cent. With these four exceptions, however, all of the 42 selections showed a degree of resistance comparable to the F_3 parent family.

Six selections also were grown from each of three susceptible F_3 families, Nos. 52, 54, and 60, from No. 1015a1. In 1919, family No. 52 showed 35.2 per cent infection. In 1920, the percentages of infection of the selections ranged from 25.2 per cent to 53.5 per cent. Family No. 54 in 1919 had 58.1 per cent of infection. In 1920, the selections showed infections ranging from 45.7 per cent to 78.5 per cent. Family No. 60 in 1919 had 38.2 per cent of infection and, in 1920, the selections showed infections ranging from 34.6 per cent to 45.8 per cent. These selections from very susceptible F_3 families showed a wider range of infection than the parents.

SMUT INFECTION AT BROOKLYN IN 1922

As noted previously, F_4 plants were grown at Brooklyn from seed harvested from the F_3 rows of the 1919 sowings, although no attempt was made to grow plants from a single F_3 parent. F_5 plants from the F_4 generation of 1920 also were grown. Separate sets of seed were inoculated with *Ustilago avenae* and *U. levis*. The results from *U. avenae* were much lower than might be expected. However, all the results obtained with this smut during that season were unusually low and a partial explanation is that the spores used showed much poorer germination than those employed in previous seasons. However, the results with *U. levis* in the general varietal experiments also were somewhat below those of previous years, indicating that the season was not especially favorable for these smuts.

In general, however, the F_3 families which had previously shown high susceptibility showed the highest infections in 1922, and the very resistant F_3 families showed very low infections or entirely negative results. In many cases, negative results were obtained with F_4 plants derived from moderately resistant F_3 families. The results, however, are in harmony with those previously obtained. The evidence also indicates that the selections react in a similar fashion to both smuts. Selections highly resistant to one smut also are resistant to the other, and selections highly susceptible to the one are susceptible to the other. In some cases the percentage of infection with *Ustilago levis* was too low, but the data obtained are not sufficient to prove whether or not there are cases in which the susceptibility of a selection to the two smuts is different.

RELATIONSHIP OF PLANT CHARACTERS TO SMUT RESISTANCE

The data on certain spikelet characters of the F_2 plants shown in Table I apparently indicate that no definite relationship or correlation exists between morphological characters and resistance or susceptibility to these smuts. (See pl. 4.)

With regard to the presence and absence of awns, it will be seen that smut resistance is not confined to those families in which the parent F_2 plant was either fully awned or practically awnless. Of the 12 F_2 plants producing immune or very resistant progeny, 7 were described as having abundant awns, 1 as being commonly awned, and 4 as having few awns. On the other hand, of the 36 F_2 plants producing moderately susceptible or very susceptible progenies, 24 were placed in the abundantly awned class, 7 in the commonly awned class (approximately 50 per cent of spikelets awned), and 5 in the class with few awns.

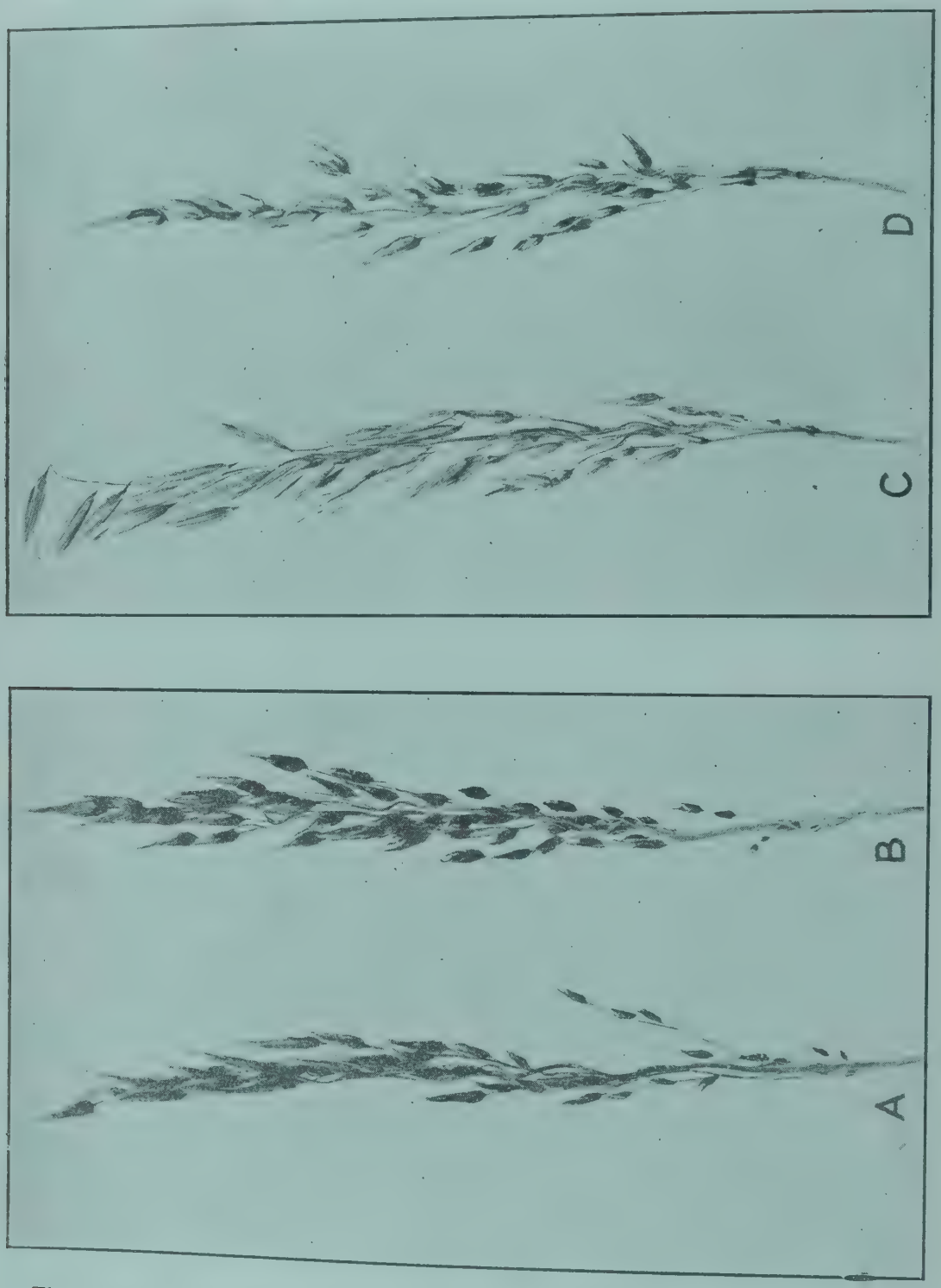
The disjunction of the second floret is discussed in terms of the disarticulation of the rachilla segment in *sativa* and its permanent attachment to the floret in *sterilis*. The 12 F_2 plants producing immune or very resistant progenies were classed as follows: 2 attached (Fulghum), 10 intermediate, and none disarticulated (Swedish Select). The classification of the 36 F_2 plants producing moderately susceptible or very susceptible progenies was as follows: 9 attached (Fulghum), 5 disarticulated (Swedish Select), and 22 intermediate.

On the basis of lemma color the 12 F_2 plants that produced immune or very resistant progenies, were described as follows: 1 reddish-yellow, 4 yellow, and 7 yellowish-white. The 36 plants producing moderately or very susceptible progenies were represented as follows: 6 reddish-yellow, 21 yellow, 9 yellowish-white.

It thus will be seen that no definite correlation appears to exist between morphological characters and smut resistance or smut susceptibility. Further evidence supporting this indication is shown by data recorded on the F_3 progenies from certain of the above very resistant and moderately susceptible or very susceptible plants which were grown in agronomic nurseries to determine their uniformity and general vigor.

For example, it was observed that, of the 14 plants grown from F_2 plant No. 1015a1–12, 6 resembled the Fulghum parent and 8 the Swedish Select parent in most characters, thus showing the genetic segregation of morphological characters. A similar observation was made on the F_3 progeny from plant No. 1015a1–33. The data in Table I show that the percentage of smut in both the F_3 and F_4 progenies from the plant No. 1015a1–12 was zero. No smut was present in the F_3 generation of plant No. 1015a1–33, but in the F_4 and F_5, 2.9 and 0.8 per cent, respectively, of the plants of one F_3 selection were infected. In the F_5 generation 2 per cent of the plants from another F_3 selection of plant No. 1015a1–33 were infected with *Ustilago levis*.

Under the environment of Aberdeen, Idaho, the Fulghum variety produces rather short culms, but the Swedish Select oat grows much taller. As a re-



The smuts of oats. A and B, loose smut, *Ustilago avenae;* C and D, covered smut, *U. levis*

sult, observations were made on the relative plant height in some of the F_3 progenies. Of the F_3 progeny of F_2 plant No. 1015a1–45, some were tall like Swedish Select, and others were short like Fulghum. Similar variations in plant height were indicated in some of the other families that proved very resistant to smut. In the very susceptible families No. 1015a1–52 and No. 1015a1–54, indications of marked variation in plant height also were observed.

These observations, therefore, indicate rather definitely that the plant characters of either parent are not correlated with smut resistance or smut susceptibility. The factors for smut resistance apparently are inherited independently of morphological characters in the cross in question (Fulghum × Swedish Select).

CONCLUSIONS

1. The behavior of 92 F_3 families of a cross between Fulghum and Swedish Select to *Ustilago avenae* and *U. levis* has been studied. A very wide range of susceptibility of the families has been noted. A large majority of the families have shown a moderate or high degree of susceptibility Twenty-five families have shown a resistance comparable to that of the resistant Fulghum parent, and eight families a susceptibility greater than that of Swedish Select.

2. The F_4 selections from susceptible F_3 families all proved to be highly susceptible, whereas the F_4 selections from resistant F_3 families in general have been very resistant. In one case, however, two of the latter selections showed rather high percentage of infection.

3. The selections appear to behave in a similar fashion toward both loose and covered smut. These results are in harmony with those obtained by Reed (*14*) and Reed, Griffiths, and Briggs (*15*) in connection with their studies on the varietal resistance of oats to loose and covered smuts. Very few varieties of oats have been found which show any marked differences in their resistance to the loose and covered smuts.

4. There appears to be no correlation between morphological characters of the various selections and their susceptibility to the smuts. Certain F_3 families with the general characters of Fulghum have been found to be highly susceptible. On the other hand, some families more or less similar to Swedish Select have proved to be highly resistant. Thus it is possible to obtain the desired combination of resistance to smut and other varietal characters of oats.

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A COMPARISON OF DIRECT AND INDIRECT CALORIMETRY IN INVESTIGATIONS WITH CATTLE [1]

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INTRODUCTION

From the time of the construction of the first respiration calorimeter by Lavoisier (*9, p. 355*)[2] until Rubner (*10 p. 135*) demonstrated the applicability of the law of conservation of energy to the vital processes of the animal body, the interest of experimenters with animal calorimeters centered mainly in the question as to whether the products of respiration fully represent the production of heat by the animal.

With the establishment of the validity of this conception began a new era in respiration calorimetry, which has been marked by the perfecting of apparatus and the standardization of methods. Since, however, the respiration calorimeter is a complicated and costly apparatus, both in construction and use, methods have been evolved for the indirect determination of the heat production, by the use of a respiration apparatus, without the calorimetric features.

For the purpose of indirect heat estimation there are now in use two principal methods, with modifications of detail, to meet special conditions as to kind of animal, length of experiment, equipment available, accuracy sought, and the particular problem in hand.

Briefly and generally, one of these methods (*2*) involves the estimation of the amounts of protein, carbohydrates, and fat oxidized in the body, and the computation of the heat production from the calorific values of these nutrients.

The amounts katabolized are computed from the oxygen consumed and carbon dioxide produced, and the nitrogen, carbon, oxygen, and hydrogen of the urine, these computations being based on the respiratory quotients (proportions of carbon dioxide produced to oxygen consumed) for the nutrients oxidized, these quotients being approximately 0.7 for fat, 0.8 for protein and 1.0 for carbohydrates.

The other method depends on the balances of nitrogen and carbon in the animal body, and the determination of the potential energy of the feed and excreta. By this method the heat production is measured by deducting from the gross energy of the feed the potential energy of the excreta and the energy equivalent of the body tissue gained (or adding the energy equivalent of tissue lost).

The more fundamental considerations as to energy metabolism having been established, a question of much importance is the comparative accuracy of the expensive and time-consuming direct-heat estimation by means of the respiration calorimeter and the relatively simple indirect-heat estimation by means of the two general procedures outlined.

As a contribution to the understanding of this problem the writer presents the following consideration of the possibilities of error in each, and the extent to which these errors affect results, the comparisons being based on the experimental work of this institute.

The respiration calorimeter at the Institute of Animal Nutrition, which has been used for more than 20 years in metabolism experiments with cattle, is, as the name implies, both a calorimeter and a respiration apparatus. In each experiment heat production has been determined directly, and also, for comparison, by computation from the balance of matter and energy, as in the second of the indirect methods referred to above. This method is essentially the same as that used by Kellner (*7*) in his extensive researches on cattle.

[1] Received for publication June 11, 1924; issued May, 1925. This study was undertaken at the suggestion of Dr. E. B. Forbes, to whom the writer is especially indebted for kindly criticism and assistance in revising the manuscript. He is also under obligations to the other members of the staff of the Institute for many valuable suggestions in connection with this work.

[2] Reference is made by number (italic) to "Literature cited," p. 406.

A comparison of the observed and computed heat production in experiments with steers, conducted in the years prior to and including 1909, has been reported by Armsby (*6*). Since the year 1909 a number of experiments have been made on steers, and on cows as well, and upon these the present paper is based.

OBSERVED AND COMPUTED HEAT PRODUCTION

Before entering upon a discussion of the direct and indirect methods of determining the heat production by animals let us compare the results obtained by the two methods. Tables I and II present the results of experiments with steers and cows, the steer experiments comprising 35 periods and the cow experiments 36. Each period was of 48 hours' duration (except in experiment 221a and 221d, 886–I, where the periods were 24 hours).[3] All results have been computed to a 24-hour basis.

The results of the steer experiments show that in 21 cases out of the 35 the computed heat production is higher than the observed; in 10 cases the computed heat is lower than the observed, and in 4 cases the difference between the two is so small as to be negligible.

Considering the differences in the individual trials, and arranging them in groups according to magnitude, the results are as follows: In 21 cases, or 60.0 per cent of the total number, the difference exceeds 1 per cent, of which 13 are plus (that is, show the computed heat production the larger); in 14 cases, or 40.0 per cent of the total number, the difference exceeds 2 per cent, of which 10 are plus; in 6 cases, or 17.1 per cent of the total number, the difference exceeds 3 per cent, of which 5 are plus; in 2 cases, or 5.7 per cent of the total number, the difference exceeds 4 per cent, of which both are plus; and in 2 cases, or 5.7 per cent of the total number, the difference exceeds 5 per cent, of which both are plus.

TABLE I.—*Observed and computed heat production of steers*

Experiment No.	Animal	Period No.	Gain by animal	Heat production in 24 hours		Difference	
				Computed	Observed		
			Calories	*Calories*	*Calories*	*Calories*	*Per cent*
210	Steer D	I	−845.7	9,262.9	9,460.6	−197.7	−2.1
		II	−1,141.5	8,026.1	8,186.0	−159.9	−1.9
		III	−2,153.8	6,942.0	7,110.4	−168.4	−2.4
211	do	I	−883.2	12,139.1	11,546.9	+592.2	+5.1
		II	−496.9	9,813.8	9,596.5	+217.3	+2.3
		III	+7,663.8	13,815.6	13,937.2	−121.6	−0.9
		IV	−2,462.1	9,227.4	9,196.5	+30.9	+0.3
		V	−4,968.6	8,010.9	7,952.9	+58.0	+0.7
211	Steer G	I	+327.9	11,642.1	11,710.8	−68.7	−0.6
		II	−1,565.5	8,229.9	8,197.0	+32.9	+0.4
		III	+6,133.6	12,807.3	13,290.2	−482.9	−3.6
		IV	−2,981.5	8,942.6	8,936.5	+6.1	+0.1
		V	−4,154.1	6,894.8	6,882.5	+12.3	+0.2
212	Steer H	I	+2,241.2	11,130.7	11,022.9	+107.8	+1.0
		II	+2,529.3	10,690.4	10,903.4	−213.0	−1.9
		III	+840.4	9,712.9	9,723.1	−10.2	0.1
		IV	+859.9	9,805.8	9,701.6	+104.2	+1.1
		V	−739.6	7,296.0	7,304.9	−8.9	−0.1
		VI	−409.8	6,950.4	6,773.1	+177.3	+2.6
216	Steer J	I	+5,111.5	15,931.6	15,541.2	+390.4	+2.5
		II	−1,046.5	9,836.3	9,547.2	+289.1	+3.0
		III	+596.7	11,420.2	10,865.8	+554.4	+5.1
		IV	−2,672.9	8,186.4	8,020.4	+166.0	+2.1
		V	+2,299.7	12,820.9	12,827.5	−6.6	0.1
		VI	+907.3	11,062.8	10,838.2	+224.6	+2.1
		VII	−1,708.8	8,501.5	8,398.4	+103.1	+1.2
217	do	I	+927.2	11,240.9	10,896.8	+344.1	+3.2
		II	+8,493.1	16,934.8	16,671.7	+263.1	+1.6
		III	+6,463.3	21,416.0	21,204.9	+211.1	+1.0
		IV	−559.6	14,716.0	14,224.4	+491.6	+3.5
220	Steer K	I	−626.0	11,881.5	12,123.6	−242.1	−2.0
		II	−2,270.5	10,108.7	10,288.1	−179.4	−1.7
		III	+464.5	10,809.7	10,754.2	+55.5	+0.5
		IV	+4,644.5	13,524.3	13,844.0	−319.7	−2.3
		V	−2,951.5	10,136.3	10,055.8	+80.5	+0.8
Totals and averages, 35 periods				379,868.6	377,535.2	+2,333.4	+0.6

[3] Period 886–I, experiment 221d, was to have covered 48 hours, but owing to the failure of the electric current the experiment was discontinued during the second 24 hours.

TABLE II.—*Observed and computed heat production of cows*

Experiment No.	Cow No.	Period No.	Dry or in milk	Gain by animal	Heat production in 24 hours		Difference	
					Computed	Observed		
				Calories	*Calories*	*Calories*	*Calories*	*Per cent*
221a	631	I	In milk	+221.4	11,720.5	11,463.9	+256.6	+2.2
		II	do	+3,663.2	12,598.8	12,333.9	+264.9	+2.2
		III	Dry	+3,863.5	13,680.3	13,219.2	+461.1	+3.5
221a	615	I	In milk	+502.0	11,756.5	11,435.5	+321.0	+2.8
		II	do	−160.7	12,286.2	12,137.3	+148.9	+1.2
		III	do	−650.3	12,747.6	12,032.2	+715.4	+6.0
221a	579	I	do	+384.7	13,545.9	13,235.6	+310.3	+2.3
		II	do	+686.7	13,165.5	12,896.2	+269.3	+2.1
221c	885	I	do	−2,883.6	11,007.1	11,221.4	−214.3	−1.9
		II	do	−2,957.5	9,023.7	8,862.4	+161.3	+1.8
		III	do	−4,830.4	9,032.1	8,859.0	+173.1	+2.0
221c	886	I	do	+2,976.8	10,183.6	11,191.7	−1,008.1	−9.0
		II	do	−1,228.2	8,228.7	8,568.6	−339.9	−4.0
		III	do	+3,018.2	10,885.9	11,063.4	−177.5	−1.6
221d	885	I	Dry	+730.6	8,201.5	8,380.3	−178.8	−2.1
		II	do	+4,410.1	9,099.0	9,629.1	−530.1	−5.5
		III	do	+366.9	9,289.5	8,816.7	+472.8	+5.4
221d	886	I	do	+897.8	8,407.9	8,036.9	+371.0	+4.6
		II	do	+4,389.2	10,774.5	10,474.5	+300.0	+2.9
		III	do	+895.5	8,511.0	7,892.4	+618.6	+7.8
221e	885	I	do	+2,625.6	9,157.4	9,721.7	−564.3	−5.8
		II	do	−155.0	8,444.7	8,716.4	−271.7	−3.1
221e	886	I	In milk	+1,795.0	11,852.0	12,047.9	−195.9	−1.6
		II	do	+1,685.3	11,136.6	11,049.3	+87.3	+0.8
221e	874	I	do	+537.3	11,563.9	11,417.0	+146.9	+1.3
		II	do	+2,998.6	11,456.6	11,512.6	−56.0	−0.5
221f	874	I	Dry	+3,500.6	11,279.2	10,964.2	+315.0	+2.9
		II	do	+159.6	9,445.2	9,076.8	+368.4	+4.1
221f	887	I	do	+2,019.0	10,649.3	10,327.2	+322.1	+3.1
		II	do	+240.0	8,733.5	8,395.0	+338.5	+4.0
221f	886	I	In milk	+26.5	13,187.1	13,589.4	−402.3	−3.0
		II	do	+2,151.0	12,268.1	12,422.0	−153.9	−1.2
221g	887	I	do	+7.0	12,701.3	12,367.7	+333.6	+2.7
		II	do	−5,327.8	8,887.9	8,627.1	+260.8	+3.0
		III	do	−827.5	10,429.9	9,870.3	+559.6	+5.7
		IV	do	+2,270.1	12,010.5	11,670.4	+340.1	+2.9
Totals and averages, 36 periods					387,349.0	383,525.2	+3,823.8	+1.0

The extreme percentage differences are +5.1 and −3.6, while the total computed heat production for the 35 trials exceeds the observed by only 0.6 per cent.

In the 57 earlier steer experiments reported by Armsby (*6*) the total observed heat production for the 57 trials differed from that computed by only 0.4 per cent, while the extreme percentage differences in individual cases were +7.6 and −5.1 per cent. Grouping the results of the 57 trials as above one finds: In 30 cases, or 52.6 per cent of the total number, the difference exceeds 1 per cent, of which 17 are plus (in favor of the computed heat production); in 21 cases, or 36.8 per cent of the total number, the difference exceeds 2 per cent, of which 13 are plus; in 14 cases, or 24.6 per cent of the total number, the difference exceeds 3 per cent, of which 10 are plus; in 6 cases, or 10.5 per cent of the total number, the difference exceeds 4 per cent, of which 4 are plus; in 4 cases, or 7 per cent of the total number, the difference exceeds 5 per cent, of which 3 are plus; in 2 cases, or 3.5 per cent of the total number, the difference exceeds 6 per cent, of which both are plus; and in 1 case, or 1.8 per cent of the total number, the difference exceeds 7 per cent, which is plus.

In general, the results of the steer experiments given in Table I compare favorably with those reported by Armsby in showing a close agreement between the observed and the computed heat production, though there are considerable differences in a few cases.

In the cow experiments the differences between the observed and the computed heat production are wider than with the steers. Of the 36 trials, 24 show a higher computed heat production than the observed, while in 12 cases the observed heat production is higher than that computed, the percentage differences ranging from +7.8 to −9.0. The total computed heat

production for the 36 periods is higher than that observed by 1.0 per cent. Grouping the results as before one has: In 34 cases, or 94.4 per cent of the total number, the difference exceeds 1 per cent, of which 23 are plus; in 26 cases, or 72.2 per cent of the total number, the difference exceeds 2 per cent, of which 20 are plus; in 14 cases, or 38.9 per cent of the total number, the difference exceeds 3 per cent, of which 9 are plus; in 9 cases, or 25 per cent of the total number, the difference exceeds 4 per cent, of which 6 are plus; in 7 cases, or 19.4 per cent of the total number, the difference exceeds 5 per cent, of which 4 are plus; in 2 cases, or 5.6 per cent of the total number, the difference exceeds 6 per cent, of which 1 is plus; in 2 cases, or 5.6 per cent of the total number, the difference exceeds 7 per cent, of which 1 is plus; and in 1 case, or 2.8 per cent of the total number, the difference exceeds 8 per cent which is minus.

In considering, then, the results of Tables I and II, the following questions suggest themselves: First, in comparing the heat production obtained by the direct and the indirect method which of the two possesses the greater accuracy? Second, what are the possible causes for the wider differences between the computed and the observed heat production in the cow experiments than in those made on steers? In studying these questions let us consider, briefly, the sources of error and the methods used in each.

SOURCES OF ERROR IN THE INDIRECT METHOD

The indirect method here considered, by which the figures for computed heat production in Tables I and II were obtained, is the second of the two indirect methods referred to above, the so-called balance method. The basic principle upon which both of these indirect methods are based is that the oxidation of a given substance in the body liberates the same amount of energy as does its oxidation outside the organism. The difference, then, between the gross energy of the feed and the energy of the total excreta, as determined by analytical methods, gives the energy derived from the feed, and is equal to the heat given off by the animal, in case the animal does not gain or lose body tissue. A loss of body tissue, of course, involves a liberation of energy exceeding that derived from the feed; therefore the energy-equivalent of the body tissue lost is added to the energy derived from the feed in computing the total heat production. On the other hand, a gain of body tissue means that not all of the energy derived from the feed was liberated, a part of it being stored in body tissue, the energy-equivalent of which is subtracted from the energy of the feed in computing the heat production.

Representing the heat production by H, the energy of the feed by F, the energy of the total excreta (feces, urine, methane, brushings, and milk) by E, and the energy of the body gain by G, the general working formula for heat production is $H=F-E-G$.

The estimation of the gain or loss of body tissue, which is an essential feature of this method, is computed in accord with the conception of the schematic body, which regards the organic matter of the animal as composed essentially of protein and fat, with at most comparatively small amounts of carbohydrates (glycogen). The supply of glycogen is assumed to remain constant during the experiment, and the gain or loss of protein and fat is estimated from a balance between income and outgo of nitrogen and carbon.

The factors used in the computation are the following: protein$=N\times 6$; fat$=C\times 1.307$; carbon in protein$=$ 52.54 per cent; heat of combustion of protein per gram$=5.7$ Calories; heat of combustion of fat per gram$=9.5$ Calories. The derivation of these factors is fully discussed by Armsby (*2*).

The analytical data needed for computing the heat production by the balance method are: (1) Dry matter in the feed, feces (or feces and urine mixture) and brushings; (2) nitrogen in the feed, feces, urine, milk, and brushings; (3) carbon in feed, feces, urine, milk, and brushings; (4) carbon in the gaseous excreta (respiration apparatus); (5) carbon in methane (by respiration apparatus); (6) energy in the feed, feces, urine, milk, and brushings.

The accuracy of this indirect method obviously depends on the accuracy of the factors used and on the accuracy of the analytical results. To what extent the factors used may be responsible for the differences between the computed and the observed heat production is difficult to say. Certainly any error due to the factors used should be least where the gain or loss of body tissue is least. Referring, however, to the results in Tables I and II, one notices in many cases relatively small differences between the observed and

the computed heat production where the body gains or losses are considerable, and large differences where the gains or losses are comparatively small. It will be noted that in these tables the net gain in calories is given, and that the differences are somewhat obscured in those cases in which there was a gain of one substance and a loss of another by the algebraic addition of their energy equivalents. However, the separate consideration of the gains or losses of protein and fat does not materially alter the general relationship referred to above, since in most cases in which there was a considerable gain or loss of body tissue both fat and protein were gained or lost together.

In those cases, at least, in which the gains or losses of body tissue are small and the differences between the observed and the computed heat production are large, if there is any serious error in the computed heat production it should be looked for in the analytical results. An examination of some of the analytical data recently obtained by the methods used in this institute has revealed possibilities of error which in the case of the experiments with cows deserve especial consideration. In these experiments there is, in addition to the problem of handling the milk, the necessity of collecting urine and feces together, thus giving rise to new difficulties in the handling and preparation of the samples for analysis.

With this exception, essentially the same experimental procedure and analytical methods were followed in the cow experiments as in the steer experiments (*5, p. 200–222*). It is important, however, to bear in mind the following points in connection with the computation of the nitrogen, carbon, and energy balances: (1) In the steer experiments the nitrogen in the feces was determined in the fresh substance, while the carbon was determined in the air-dried sample, by means of a combustion furnace. (2) In the urine the carbon was determined in the fresh material, while the nitrogen was determined both in the fresh material and in a sample dried in the vacuum desiccator in the same manner as for energy determination. From the nitrogen loss thus determined, in drying, an energy correction was computed as applying to the energy determined by the bomb, assuming that the loss of nitrogen in drying represented a loss of urea and a corresponding loss of energy.

In the cow experiments (except in 221a) [4] the feces were collected together with the urine. The nitrogen was determined in the composite of daily samples both in the fresh and in the air-dried materials in all cases, in order to ascertain the loss during drying. Carbon was determined in the air-dried sample by means of the bomb, in all cases, and also (in experiments 221g and 221f) in the fresh substance, by means of an electric combustion furnace. An attempt was made to determine the carbon in the fresh sample of the feces-and-urine mixture in the first of the experiments with cows, but owing to lack of laboratory help during the war and for some time thereafter, and to the initial technical difficulties with the method, no satisfactory determinations of the carbon in the fresh feces-and-urine mixture are available for the earlier cow experiments. The energy of the feces-and-urine mixture was determined in the air-dried sample.

The air-drying was conducted by spreading the material out on trays in a drying closet in which it was exposed to a current of air heated to about 60° C. by passage over a steam coil. To hasten the drying the material was carefully broken up, when partly dried, by means of a spatula. When thoroughly dry, the material (properly protected) was exposed to the air of the grinding room for several days. It was then weighed, ground as rapidly as possible, and preserved for analysis in sealed, glass-stoppered bottles. In this weighing and grinding especially dry or especially damp days were avoided.

LOSS OF NITROGEN AND CARBON IN AIR-DRYING FECES-AND-URINE MIXTURE

A comparison of determinations of the daily nitrogen and carbon of the feces-and-urine mixture, in the fresh samples, with determinations on air-dried samples, revealed the fact that a considerable loss of these constituents occurred during the process of drying, and that the loss of carbon exceeded the quantity required to combine with the nitrogen lost to form either urea or ammonium carbonate. Table III exhibits these losses as occurring in experiments 221f and 221g.

The figures in Table III show clearly that the carbon in the air-dried feces and urine is far from the original total, even after adding to it the carbon-equivalent of the nitrogen lost, computing this nitrogen to urea or ammonium carbonate.

[4] In experiment 221a the feces and urine were collected together, in the calorimeter, while during the other days of the digestion period they were collected separately.

TABLE III.—*Loss of nitrogen and carbon during the drying of cow's feces-and-urine mixture*

Experiment and period	Dry matter per day	Nitrogen lost per day	Carbon lost per day		
			Total	Combined with nitrogen computed from N lost)	Not combined with nitrogen
221f:	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
874 I	2,225.8	78.3	71.9	33.6	38.3
874 II	1,516.0	50.3	58.8	21.6	37.2
887 I	1,887.4	82.0	70.7	35.2	35.5
887 II	1,296.5	59.2	48.5	25.4	23.1
886 I	3,178.8	83.9	119.6	36.0	83.6
886 II	2,739.8	96.8	124.7	41.5	83.2
221g:					
887 I	2,793.1	57.7	108.3	32.5	75.8
887 II	1,405.3	58.5	82.2	25.1	57.1
887 III	2,072.2	61.4	79.3	26.3	53.0
887 IV	2,924.1	87.3	83.5	37.5	46.0

To diminish this possibility of error in the carbon balance, in those cow experiments in which the carbon was not determined in the fresh feces-and-urine mixture, the loss of carbon on drying, per gram of nitrogen lost, was computed from the average of the data available, this average being 1.15 gm. and the correction for carbon lost on drying was computed by the use of this factor. That this correction is not entirely satisfactory is realized, since the loss of carbon does not in all cases follow exactly the loss of nitrogen, but it was considered, under the conditions, as the best possible way of correcting these earlier results which were obtained from the air-dried material.

LOSS OF ENERGY IN AIR-DRYING FECES-AND-URINE MIXTURE

A loss of nitrogen and carbon on drying does not mean a loss of only these elements, but it also represents a certain loss of potential energy. While the original fresh feces-and-urine mixture might have contained some carbon as free CO_2, the quantity so contained, in view of its solubility, could be only a very small fraction of the total quantity lost during the drying. Some of this loss no doubt came from the decomposition of urea, and this portion has been estimated in the experiments from the nitrogen lost. The remainder has been assumed, in the absence of definite knowledge, to be a result of carbohydrate fermentation, since the conditions of the drying are regarded as favorable for such a process. On this basis the energy lost on drying has been computed by using Rubner's factor, 5.45 for loss in Calories per gram of nitrogen lost, and the factor 9.4 for computing Calories per gram of carbon lost in excess of that required to satisfy the nitrogen lost, the latter factor representing the energy per gram of carbon in starch.

Although the above computation is not without logical justification, one must admit the possibility of considerable error in this correction, in view of our imperfect knowledge as to the nature and extent of the losses. Since it is impracticable directly to determine the energy in the fresh feces-and-urine mixture, the drying process may be, therefore, a source of serious error in the computation of the heat production.

To illustrate the extent to which the above considerations, as to losses and corrections may affect the computed heat production Table IV has been compiled, the data in the three methods of computation being derived from the data of experiments 221f and 221g. In all three cases the nitrogen was determined in the fresh substance, and energy in air-dry substance. In Method I carbon was determined in the air-dry material. Corrections for carbon and energy were computed from the nitrogen lost, calculated as urea. In Method II carbon was determined in the fresh material, corrections for energy being computed as in Method I. In Method III the carbon was determined in the fresh material and the corrections for energy were computed as in Method I, with an additional correction for carbon lost in excess of that required to combine with the nitrogen to form urea (using the factors 5.45 per gram nitrogen and 9.4 per gram carbon).

TABLE IV.—*Computed heat production as affected by loss of carbon during drying*

Experiment No.	Cow No.	Period No.	Method I. Carbon of air-dry substance; carbon and energy corrected for loss of N		Method II. Carbon of the fresh substance; energy corrected for loss of N		Method III. Carbon of the fresh substance; energy corrected for loss of N and for C uncombined with N	
			Computed heat production	Computed ÷ observed	Computed heat production	Computed ÷ observed	Computed heat production	Computed ÷ observed
			Calories	*Per cent*	*Calories*	*Per cent*	*Calories*	*Per cent*
221f	874	I	11,163.7	101.8	11,639.2	106.2	11,279.2	102.9
		II	9,334.0	102.8	9,794.9	107.9	9,445.2	104.1
221f	887	I	10,542.4	102.1	10,983.2	106.4	10,649.5	103.1
		II	8,663.8	103.2	9,142.6	108.9	8,733.4	104.0
221f	886	I	12,923.8	95.9	13,972.1	102.9	13,176.0	97.0
		II	12,017.1	96.7	13,050.2	105.1	12,268.1	98.8
221g	887	I	12,472.7	100.8	13,413.8	108.5	12,701.3	102.7
		II	8,715.6	101.0	9,424.6	109.2	8,887.9	103.0
		III	10,270.0	104.0	10,928.1	110.7	10,429.9	105.7
		IV	11,871.8	101.7	12,442.9	106.6	12,010.5	102.9

In consideration of the loss of carbon on drying the feces-and-urine mixture, the results of Method I are seemingly paradoxical. Why, one may ask, by using a figure for carbon in the feces and urine, known to be much lower than the correct value, does the computed heat production agree closely with the observed, in fact, in most cases, even more closely than when computed by Method III? The answer lies in the details of the computation. Referring to the general formula for heat production, $H=F-E-G$, it is seen that H remains unchanged if G is increased and E is decreased simultaneously by the same amount. When, in computing the balance of carbon, the carbon lost through fermentation is ignored, the figure for carbon in the excreta is low, and the apparent gain of carbon and, therefore, of energy (G) is correspondingly high or the loss low. On the other hand, if one ignores that portion of energy of the feces and urine which was lost in drying as a result of the fermentation, the energy of the feces and urine (and therefore E) is low. These two factors, therefore, oppose each other, the net effect being the difference between the two. For example, in experiment 221f, 874–I, the carbon calculated to have been lost from the excreta by fermentation was 38.3 gm. This being ignored, the balance of carbon showed a gain which was 38.3 gm. too high. This is equivalent to 50.06 gm. of fat (38.3×1.307), or in terms of energy, 475.5 Calories (50.06×9.5). G is therefore 475.5 Calories too high. The heat lost in fermentation, computed from the carbon, was 360.0 Calories. This being ignored, E is too low by 360.0 Calories. The net effect on the computed heat production is therefore $360.0-475.5=-115.5$ Calories, which accounts for the difference between I and III in Table IV ($11,279.2-11,163.7=115.5$).

To state this somewhat differently, since the metabolizable energy equals the gross energy of the feed minus the energy of the feces, urine, and methane, when the metabolizable energy and the energy of the body gain are increased simultaneously the net effect on the computed heat production equals the increase of the former minus the increase of the latter. Thus, in the case just cited, the metabolizable energy is too great by 360 Calories, while the energy of the body gain is too great by 475.5 Calories. The net effect on the computed heat production is, therefore, $360.0-475.5=-115.5$ Calories.

Thus it is possible to obtain a figure for heat production, which may agree closely with the observed, as a result of a balance of opposing errors.

The results of Method II show unusually large differences between the computed and the observed heat production, the carbon in the feces and urine as determined in the fresh substance being used, and the energy lost in fermentation ignored.

The results of Method III are as they appear also in Table II. The basis for the computation and the possibilities of error have already been considered.

The results as set forth in Tables III and IV indicate the need of rigorous control of the conditions of drying the feces and urine, and further investigation of the nature of the material lost during drying.

Recurring now to the computation of the heat production in the steer experiments, the feces and urine were collected, dried, and analyzed separately. The carbon in the urine was determined in the fresh sample, and the correction for energy lost on drying was computed from the nitrogen lost. The carbon in the feces was, however, determined in the air-dried sample, ignoring the possibility of loss of carbon and energy during the drying. There is no data covering the losses on drying steer feces. There is, however, data which show a considerable loss of carbon in drying cow feces (not a mixture of urine and feces), which also implies a loss of energy. The results of the analysis of several samples of cow feces, which were collected by an attendant on a single day of the digestion experiments, are given in Table V.

POSSIBLE ERROR IN THE DETERMINATION OF DRY MATTER OF FECES-AND-URINE MIXTURE

The determination of the dry matter of the feces-and-urine mixture is another source of error to which attention is called.

For determinations of energy, nitrogen, carbon, etc., in the combined feces and urine, the composite sample representing several days' collection was used. In all cow experiments this composite was made up of 10 daily aliquots. The dry matter, however, was determined in this composite and also in each of the daily samples. The same routine was followed in the drying of the daily samples as in the case of the composite, except that the former were generally left in the drying closet for a longer period of time, since, as a rule, they were placed in the closet as soon as obtained, and were allowed to remain until the composite sample also was thoroughly dry.

In all cases but one the percentage of dry matter as determined on the composite was found to be higher than the true average of determinations on the daily samples. Such a uniform difference must have an explanation other than errors of sampling or weighing. Aside from these errors, such differences could be due either to an unweighed evaporation of moisture from the composite during preparation, or to a greater loss of dry matter during the drying of the daily samples. Considering the precautions taken to prevent evaporation the loss by this means could have been but slight. The loss of dry matter due to fermentation

TABLE V.—*Losses of nitrogen and carbon occurring during the drying of cow feces*

Experiment and period	Dry matter of feces	Loss of nitrogen		Loss of carbon		Loss of carbon per gram nitrogen lost
	Grams per day	*Per cent* [a]	*Grams per day*	*Per cent* [a]	*Grams per day*	*Grams*
221f:						
874 I	2,013.7	0.092	1.86	1.045	21.0	11.29
874 II	1,409.7			1.208	17.0	
887 I	1,560.2	.367	5.73	3.379	52.7	9.20
887 II	1,086.3	.240	2.61	1.891	20.6	7.89
886 I	2,762.2	.236	6.53	1.872	51.7	7.92
886 II	2,796.0	.208	11.41	3.114	87.1	7.63
221g:						
887 I	2,755.4	.165	4.54	1.672	46.1	10.15
887 II	1,147.2	.124	1.43	1.000	11.5	8.04
887 III	1,583.4	.160	2.53	1.384	21.9	8.66
887 IV	2,665.1	.096	2.56	3.228	86.3	33.71

[a] Computed to dry-matter basis.

These results show that while the loss of nitrogen is slight, the loss of carbon is several times as great.

It would, of course, be unsafe to assume that identical losses occurred in the drying of steer feces since the composition of the rations, and, therefore, presumably, of the feces differed from that applying to the experiments with cows. Furthermore, the losses on drying are shown to be too variable to be regarded as accurately applylng to other conditions. However, the data suggest the probability of significant loss of carbon during the drying of steer feces also.

The bearing of the facts as to losses on drying, as disclosed by the above data, is also obviously important in connection with digestion experiments.

during the drying of the daily samples appears, therefore, to be the more extensive factor of error, but since there must have been some error on both these accounts the true figure for dry matter must have been higher than the one and lower than the other estimation, and there being no means of determining this intermediate figure exactly, the average of the two was used.

Table VI gives the results of the determinations of dry matter in the composite sample, the average of determinations in the daily samples, and the average of the two, as well as the possible error, in per cent and in grams of dry matter per day.

The differences between the two percentages of dry matter are fairly uniform. The average of all differences (omitting that of experiment 221c, 886 I) is 0.869 per cent. This means that, on the average, the dry matter as determined on the composite is about 0.9 per cent higher than the true average of the daily determinations or that the possible error of using the average of the two is about ±0.4 per cent. The magnitude of the error in grams per day obviously depends on the fresh weight of the material, and ranges in these experiments from ±14.4 gm. to ±113.2. It should be understood that the data presented apply only to the particular conditions and procedures which prevailed in this work.

An error in the determination of dry matter involves corresponding errors in energy, nitrogen, and carbon as determined on the dried material, all being in the same direction. These possible errors have been computed in Table VII, using the data from experiments 221f and 221g as examples. The possible errors in energy range from ±135.4 Calories to ±451.9 calories; the possible errors in nitrogen are small, ranging from ±0.59 gm. to ±2.46 gm.; the possible errors in carbon range from ±13.49 gm. to ±44.96 gm.

TABLE VI.—*Possible error of determinations of dry matter in feces-and-urine mixture*

Experiment and period	Fresh weight	Dry matter			Possible error	
		I Composite sample	II True average of daily samples	III Average of I and II		
	Grams	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Grams dry matter*
221c:						
885 I	20,038.4	13.182	12.312	12.747	±0.435	±87.2
885 II	14,044.4	13.170	11.946	12.558	± .612	±86.0
885 III	14,785.4	15.050	13.810	14.430	± .620	±91.7
886 I	17,137.8	12.595	15.097	13.846	∓1.251	∓214.4
886 II	12,121.0	13.044	12.242	12.643	± .401	±48.6
886 III	17,364.2	15.973	14.669	15.321	± .652	±113.2
221d:						
885 I	9,415.2	14.740	13.848	14.294	± .446	±42.0
885 II	16,614.8	12.268	11.520	11.894	± .374	±62.1
885 III	10,410.8	13.391	12.555	12.973	± .418	±43.5
886 I	9,258.9	15.511	14.523	15.017	± .494	±45.7
886 II	15,567.8	14.429	13.715	14.072	± .357	±55.6
886 III	10,464.6	13.178	12.468	12.823	± .355	±37.1
221e:						
885 I	11,784.9	----------	14.098	----------	----------	----------
885 II	8,937.8	13.954	13.094	13.524	± .430	±38.4
886 I	17,992.2	15.942	14.956	15.449	± .493	±88.7
886 II	15,846.1	15.681	15.007	15.344	± .337	±53.4
874 I	14,513.1	17.373	16.263	16.818	± .555	±80.6
874 II	11,900.2	17.099	16.857	16.978	± .121	±14.4
221f:						
874 I	13,370.7	17.131	16.163	16.647	± .484	±64.7
874 II	9,018.0	17.362	16.206	16.784	± .578	±52.1
887 I	13,424.0	14.514	13.588	14.051	± .463	±62.2
887 II	8,909.0	14.992	14.110	14.551	± .441	±39.3
886 I	20,344.0	16.073	15.099	15.586	± .487	±99.1
886 II	18,668.0	14.830	14.510	14.670	± .160	±29.9
221g:						
887 I	16,924.0	16.903	15.965	16.434	± .469	±79.4
887 II	10,220.0	14.178	13.322	13.750	± .428	±43.7
887 III	14,022.0	15.126	14.416	14.771	± .355	±49.8
887 IV	18,531.0	16.102	15.442	15.772	± .330	±61.2

TABLE VII.—*Effect of possible error in the dry matter of feces-and-urine mixture on the energy, nitrogen, carbon, and computed heat production*

Experiment and period	Possible error in daily dry matter (from Table VI)	Energy per gram dry matter	Carbon in dry matter	Nitrogen in dry matter	Possible error in energy	Possible error in carbon	Possible error in nitrogen	Energy equivalent to the carbon C×9.4	Energy equivalent to the nitrogen N×5.45	Energy equivalent to both N and C	Possible error in computed heat production
221f:	*Grams*	*Calories*	*Per cent*	*Per cent*	*Calories*	*Grams*	*Grams*	*Calories*	*Calories*	*Calories*	*Calories*
874 I	±64.7	4.425	43.801	2.845	±286.3	±28.34	±1.84	±266.4	±10.0	±276.4	∓9.9
874 II	±52.1	4.381	43.648	2.975	±228.3	±22.74	±1.55	±213.8	±8.4	±222.2	∓6.1
887 I	±62.2	4.451	44.207	2.397	±276.9	±27.50	±1.49	±258.5	±8.1	±266.6	∓10.3
887 II	±39.3	4.394	43.890	2.590	±172.7	±17.25	±1.02	±162.2	±5.6	±167.8	∓4.9
886 I	±99.1	4.560	45.368	2.485	±451.9	±44.96	±2.46	±422.6	±13.4	±436.0	∓15.9
886 II	±29.9	4.528	45.101	1.989	±135.4	±13.49	±0.59	±127.3	±3.2	±130.5	∓4.9
221g:											
887 I	±79.4	4.555	45.469	2.557	±361.7	±36.10	±2.04	±339.3	±11.1	±350.4	∓11.3
887 II	±43.7	4.547	45.303	2.464	±198.7	±19.80	±1.08	±186.1	±5.9	±192.0	∓6.7
887 III	±49.8	4.493	45.123	2.360	±223.8	±22.47	±1.18	±211.2	±6.4	±217.6	∓6.2
887 IV	±61.2	4.545	45.323	2.258	±278.2	±27.74	±1.38	±260.8	±7.5	±268.3	∓9.9

It will be noted that the possible errors in energy of the feces-and-urine mixture, as a result of the possible errors in dry matter, appear to be appreciable, and they would remain so if the nitrogen and, especially, the carbon as determined on the dried substance were not used as a basis for computing the loss of nitrogen and carbon on drying and of an energy correction corresponding to these losses.

The differences between the nitrogen and carbon as determined on the dry substance and those determined on the fresh represent the loss on drying. A positive error in the carbon and nitrogen determined on the dried substance would, therefore, mean a negative error in the computed loss on drying and, consequently, a negative error in the computed energy correction when such a correction is applied, and vice versa. As a result of this, the initial error in energy is reduced by the energy equivalent of the error in the computed loss of nitrogen and carbon during drying. These energy equivalents and the net effect on the computed heat production are shown in Table VII. The resultant error is negligible according to this method of computation. For example, if the error in dry matter in experiment No. 221f, 874I were +64.7 gm. (see Table VII), the energy of the daily feces-and-urine mixture, as computed by multiplying grams dry matter by the number of Calories per gram, would be too great by the amounts indicated in column 5, namely 286.3 Calories (64.7 times 4.425). Similarly, the figures for carbon and nitrogen as determined on the dry substance would be too high by the amounts indicated in columns 6 and 7 respectively (64.7 times $\frac{43.801}{100}$=28.34 gm. carbon and 64.7 times $\frac{2.845}{100}$=1.84 gm. nitrogen). On the other hand, by subtracting the too large amounts of carbon and nitrogen in the dry material from those determined on the fresh the results for loss of carbon and nitrogen on drying, thus obtained, would be too small by the same amounts given in columns 6 and 7. The energy corrections based on these losses would then be too small by the amounts indicated in columns 8 and 9, totaling in the case cited 276.4 Calories (column 10), and making the final error in the energy of these excreta equal 286.3−276.4=9.9 Calories, or in the computed heat production, −9.9 Calories.

DETERMINATION OF DRY MATTER IN FEED

It is obvious that an error in the determination of the dry matter of the feed would directly affect its energy equivalent, and also the carbon and nitrogen balances.

Dry matter is determined by first subjecting the sample to a preliminary determination of the air-dry substance; the sample is then ground, and the remaining hygroscopic moisture is determined in the ground sample. If there is any loss of moisture during the grinding, this is not accounted for in the determination, and would increase the figure for dry matter in the feed.

The effect of grinding on the dry matter determination is a very variable factor, the exact magnitude of which is unknown, but a preliminary investigation of this problem indicates that the

grinding of the samples may be a source of appreciable error in the dry matter determination. However, the effect of an error in the dry matter of the feed, on the computed heat production, would be relatively slight, for the reason that the metabolizable energy and the energy of the body gain would be affected in the same direction. This is illustrated by the following computation, the basis being arbitrarily assumed figures, and the effect on the nitrogen balance being disregarded:

Daily ration	8,000 gm. air-dry matter.
Error in dry matter	+0.5 per cent.
Energy per gram dry matter	4.5 Calories.
Carbon in dry matter	45 per cent.
Error in the daily dry matter	+40 gm.
Error in the metabolizable energy	40×4.5=+180 Calories.
Error in carbon balance	+18 gm.
Error in energy of the body gain	18×1.307×9.5=+223 Calories.
Error in computed heat production	180−223=−43 Calories.

OTHER SOURCES OF ERROR

THE GLYCOGEN CONTENT OF THE BODY.—The computation of the energy equivalent of the gain by the animal assumes that the glycogen content of the animal remains unchanged. With a submaintenance ration, or in the case of milking cows, when the ration is not sufficient fully to support the milk production, the possibility of a continuous loss of glycogen for some time may be considered. In the event of such loss the estimate for the energy equivalent of the body loss may be too large. In those cases in which there is a gain of body substance, after a considerable number of days of preliminary feeding on the ration of the experimental period to follow, the possibility that the glycogen content of the animal will come into equilibrium with the ration is greater, and the error involved in the assumption of a constant glycogen content of the body may not be appreciable.

CARBON EQUIVALENT TO THE NITROGEN LOST BY THE BODY.—Whether nitrogen be gained or lost by the body, it is considered to be accompanied by such an amount of carbon as is contained in an equivalent amount of protein, carbon being considered as constituting 52.54 per cent of the protein. The gain or loss in fat is computed from the carbon balance only after the carbon content of the protein gained or lost has been set aside. In case of a loss of protein, if there is a utilization of a part of the nonnitrogenous fraction of the katabolizing tissue protein the amount so utilized is not accounted for, and constitutes an error in the computation of the carbon equivalent to the nitrogen lost and, likewise, in the carbon balance.

CARBON DIOXIDE OF THE WATER CONSUMED.—The carbon dioxide content of the water consumed by the animal is usually not taken into consideration. This introduces a small error into the carbon balance, tending to reduce the apparent gain of carbon and to make the computed heat production too great.

HEAT OF HYDRATION AND SOLUTION.—Another small error is introduced in the determination of the energy of the visible excreta by neglecting the heat of hydration and solution. If heat is absorbed when solids come in contact with water, the reverse must be true, that is, heat is evolved when water is driven off. Conversely, if heat is evolved when solids are brought in contact with water, this heat must be absorbed on driving off the water. Thus, it is known that when urea is brought into solution an absorption of heat takes place, while when proteins are brought into contact with water an evolution of heat takes place. The feed is ordinarily given to the animal in an air-dry condition. Hydration and solution take place in the animal body, and corresponding evolution and absorption of heat must result. These processes may be considered as factors in the work of digestion. The energy of the urine, feces, and milk is determined after these substances have been dried. The heat of hydration and solution is not accounted for in the determination.

SUMMARY OF OBSERVATIONS ON SOURCES OF ERROR IN THE INDIRECT METHOD

Errors on the following accounts are either unavoidable or are of negligible magnitude: Possible change in the glycogen content of the body of the animal; possible utilization of the nonnitrogenous portion of the katabolized protein molecule; and the energy of hydration and solution.

It seems possible that errors due to the two factors first mentioned might be of appreciable importance in experiments in which the subjects are under submaintenance conditions.

A small determinable error is introduced by the carbon dioxide of the drinking water.

By far the most serious possibilities of error seem to lie in the methods of preparing the samples of feed, feces,

and urine for analysis. Of these the drying of the feces-and-urine mixture is the most important, since it involves the computation of corrections, the bases for which are imperfectly understood.

DIRECT HEAT MEASUREMENT

The direct measurement of the heat given off by the animal body is accomplished by means of the respiration calorimeter. Descriptions of such an apparatus, as used at this institute, and also of the methods employed, have appeared in several publications by Armsby and Fries (*3; 4, p. 263–270; 1; 5, p. 200–222*). For the present purpose the more important details may be condensed into the following:

The respiration calorimeter used at the Institute of Animal Nurtition of the Pennsylvania State College is of the Atwater-Rosa type, consisting of a Pettenkofer respiration apparatus, the chamber of which is also an animal calorimeter.

The respiration chamber is built with a double metallic wall inclosing a 3-inch dead-air space, surrounded, at a distance of 4 inches, by a wooden wall, and this in turn, at a distance of 4 inches by a second wooden wall, thus forming two air-spaces surrounding the chamber. By the heating or cooling of these air spaces the inner wall is maintained adiabatic.

The sensible heat given off by the animal, which constitutes the greater part (about three-fourths) of the total heat production, is absorbed by a current of cold water passing through copper pipes at the top of the respiration chamber, the exposure of these pipes to the air within the chamber being regulated by means of shields which can be raised or lowered by the operator. By this means the rate of removal of heat from the chamber is adjusted as required for the maintenance of an approximately constant temperature.

The temperature of the in-going air is maintained equal to that of the outcoming.

The temperature of the in-going and outcoming water is read every four minutes by means of two mercurial thermometers, graduated to 1/50° C., and carefully calibrated.

The volume of water passing through the calorimeter is measured by means of two copper meters of 100-liter capacity.

For each period of uniform water flow the product of the amount of water passing through the heat absorbers multiplied by the average temperature difference equals the amount of heat removed from the chamber in the water current.

Since a part (approximately one-fourth) of the total heat given off by the animal is in the form of latent heat of water vapor, this portion of heat, not being directly measurable by the calorimeter, is calculated from the amount of water vapor given off, using 0.587 Calorie per gram at 18° C. This heat is added to the heat removed in the water current to get the total heat emission.

CORRECTIONS

In order accurately to determine the amount of heat produced by the animal several corrections are made, for possible errors in the readings, for heat measured by the apparatus but not coming from the animal, and for heat withdrawn from the chamber but escaping measurement. The following is the list of corrections usually made:

(*a*) Corrections for difference of pressure on the bulbs of the two thermometers.

(*b*) Correction for friction of water in absorbers.

(*c*) Correction for lag in the rise or fall in temperature of the water from that at the inlet to that at the outlet of the absorber system.

(*d*) Correction for change in temperature of the absorber system during the experiment.

(*e*) Correction for heat developed by the blades of the fan used to stir the air in the calorimeter.

(*f*) Correction for change in temperature of the walls of the chamber during the experiment.

(*g*) Corrections for heat introduced into the apparatus or withdrawn from it, in feed, drink, excreta, and vessels containing these materials.

(*h*) Correction for the metabolism of the man entering the chamber during the experiment, to milk the cow.

(*i*) Correction for condensation of water on the absorber system.

(*j*) Correction for storage or loss of heat due to gain or loss of matter by the animal body during the experiment.

ACCURACY OF THE DIRECT METHOD

This list of the corrections serves to indicate the degree of accuracy which it is sought to attain. There are, however, two other sources of error which have been disregarded

because no practicable methods of measuring or estimating corrections have been found.

These errors depend on changes of body temperature of the animal during an experiment, and change in amount of moisture upon the walls of the chamber during an experiment.

Assuming, under the conditions of the experiments, the possibility of a change of ±0.6° F. (*8, p. 24-26*) (0.3° F. per day, in the same direction during a two-day period), the possible error in the daily heat determination, in the case of an animal weighing 400 kg., would be ±53 Calories.

As to the mosture factor, there is the possibility of a deposit of moisture on the walls of the chamber, or of an evaporation of moisture from the same, during the experiment, thus constituting an error in the measurement of the latent heat of water vapor produced. Under ordinary conditions this error is probably not appreciable. Its magnitude and direction would depend on the initial humidity in the chamber, length of the preliminary period, rate of ventilation, and water vapor given off by the animal. It is apparent that this error would be greater in short-time experiments than in those of longer duration.

As regards the ultimate accuracy of the direct method, from a consideration of the results of the 18 alcohol check tests reported from this institute by Armsby and Fries (*5, p. 200–222*) the authors came to the conclusion that the results of a single experiment with the respiration calorimeter may be regarded as accurate to within approximately the following percentages of the amounts determined: Heat, 1 per cent; water, 6.0 per cent.

The determination of the carbon dioxide production, which is used in the indirect method, is regarded as accurate to within approximately 0.5 per cent of the amount determined.

The results of 14 alcohol checks made subsequent to the above-mentioned report, in practically the same manner as before, and using heat values for anhydrous alcohol ranging from 7.07537 to 7.13107 Calories per gram, show also a close agreement between the computed and the observed values, the average percentages recovered being as follows: Heat 98.9 per cent; water, 108.0 per cent; carbon dioxide, 99.9 per cent. The results of these 14 individual tests are set forth in Table VIII.

It will be noted that in all these tests the observed heat is less than the computed. This rather uniform difference may be due either to the assignment of too high a heat value to the alcohol, or to the heat measurement, or to both. The range of the differences, however, is narrow. Considering the results of all the alcohol checks, and the fact that the possibility of error in short-time experiments is greater than in experiments of longer duration, the conclusion stated above, with regard to the accuracy of the direct heat measurement as well as to the carbon dioxide determination, seems to be warranted.

TABLE VIII.—*Results of alcohol check tests*

Date	Number of hours	Heat			Carbon dioxide			Water		
		Observed	Computed	Observed ÷ computed	Observed average of aspirator and meter	Computed	Observed ÷ computed	Observed average of aspirator and meter	Computed	Observed ÷ computed
		Calories	*Calories*	*Per cent*	*Grams*	*Grams*	*Per cent*	*Grams*	*Grams*	*Per cent*
Nov. 23, 1911	8	4,085.4	4,144.7	98.6	1,097.0	1,110.8	98.8	732.6	739.3	99.0
Dec. 31, 1912	8	4,252.6	4,302.0	98.9	1,170.1	1,152.9	101.4	852.2	770.0	110.7
Apr. 29, 1913	7	3,778.9	3,817.2	99.0	1,022.4	1,023.0	99.9	759.9	683.2	111.2
Dec. 4, 1913	7	3,426.8	3,491.4	98.2	928.8	935.7	99.3	679.3	631.9	107.5
Apr. 28, 1914	7	3,397.9	3,440.9	98.8	921.1	922.1	99.9	698.9	622.8	112.2
Dec. 17, 1914	7	3,481.5	3,507.7	99.3	939.5	940.1	99.9	671.8	634.9	105.8
June 23, 1915	7	3,462.8	3,478.3	99.6	931.1	932.2	99.9	689.4	629.3	109.6
May 9, 1916	7	3,507.9	3,584.5	97.9	965.1	960.6	100.5	673.0	647.4	104.0
Dec. 14, 1917	7	3,294.8	3,331.0	98.9	897.2	898.3	99.9	651.6	606.4	107.5
Jan. 3, 1918	7	3,252.1	3,310.5	98.2	884.4	893.5	99.0	637.1	602.3	105.8
Apr. 20, 1920	8	3,534.0	3,540.8	99.8	[a] 955.8	956.4	99.9	[a] 744.9	644.6	115.6
Apr. 21, 1921	8	3,383.4	3,390.7	99.8	[a] 908.4	915.9	99.2	[a] 672.1	617.2	108.9
Apr. 20, 1922	8	3,429.6	3,461.1	99.1	945.0	934.9	101.1	[a] 676.0	630.1	107.3
Nov. 24, 1922	8	3,495.2	3,542.3	98.7	949.4	949.6	100.0	685.7	637.7	107.5
Total		49,782.9	50,343.1		13,515.3	13,526.0		9,824.5	9,097.1	
Average		3,555.9	3,595.9	98.9	965.4	966.1	99.9	701.8	649.9	108.0

[a] By aspirator only.

SUMMARY OF DIRECT AND INDIRECT HEAT ESTIMATION

The results of the study of the details of direct and indirect calorimetry in experiments with steers and cows at this institute may be summarized as follows:

The direct heat measurement is in general more accurate than the balance method of indirect heat computation. The direct method, therefore, serves as a valuable check on the accuracy of the analytical work involved in the indirect method.

In the measurement of the nutritive values of feeds, however, the direct and the indirect methods are about equally accurate, and the latter is much the more easily accomplished.

One source of error, not affecting direct heat measurement, but involved in the utilization of both direct and indirect heat measurements in the determination of the net energy values of feeds, is the loss of moisture of feeds and feces during grinding preparatory to analysis, but this factor affects both methods.

The most important source of error affecting direct and indirect calorimetry in different ways is loss of matter, from the urine and feces, presumably through fermentation, during drying, preliminary to the determination of energy by means of the bomb calorimeter.

This loss, which contains carbon and nitrogen, is an important factor in the computation of the heat production by the indirect method, and in the application of the results of the direct method in determining the net energy value of feeds.

This loss of matter affects the estimation of (*a*) metabolizable energy, as determined by subtracting from the energy of the feed the energy of the excreta, (*b*) heat production, as determined by subtracting from the metabolizable energy the energy of the gain (carbon and nitrogen balances multiplied by factors), (*c*) energy of gain, as determined by subtracting from the metabolizable energy the directly observed heat production.

The determination of the energy of the gain from the nitrogen and carbon balances does not involve the loss under discussion, since the nitrogen and the carbon can be determined on the fresh substance. When, however, the direct heat estimation is used in the final computation of net energy any such advantage as might accrue from the greater accuracy of this direct heat estimation is lost through the fact that in order to derive the amount of the energy of the gain, which is a factor of the net energy estimation, the directly observed heat production must be subtracted from the metabolizable energy, this last datum being directly affected by the loss in drying of the excreta.

In those cases in which there is a gain of body tissues, or no appreciable loss, the error due to possible change in glycogen content of the body may be considered as negligible, and the estimation of the gain in energy, by the indirect method, may be as accurate as when determined by the direct method.

On account of the possibilities of change in the glycogen content of the bodies of cows, in connection with milk production, the chances for error in indirect calorimetry with cows seem greater than with steers.

There is need for careful control of the conditions of drying feces and urine, and of a thorough investigation into the composition and nature of the substances lost, in order to make possible the more accurate estimation of the dry matter and the energy of these excreta.

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THE LIFE HISTORY OF PILACRE FAGINEA (FR.) B. & BR.[1]

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INTRODUCTION

Pilacre is a fungus holding an important place in mycology, primarily on account of Brefeld's contribution to its life history and relationship. We may not agree with him as to the lines of evolution which he lays down, nor be able to confirm all his work as to questions of fact in each case. No one, however, has given a finer illustration of the kind and quality of work to be done in support of a theory than did Brefeld in his study of "*Pilacre petersii* B. & C." The writers have followed the life history of this "primitive basidiomycete" in pure cultures. In addition to confirming in the main Brefeld's work, as far as he was able to carry his investigation, the writers here give additional information which should be helpful in clearing up the question of the relationship of *Pilacre faginea* (Fr.) B. & Br. to the great groups of fungi.

Basing his argument on the old doctrine that, so far as structures in fruiting organs are concerned, evolution proceeds from the indefinite to the definite, and from larger numbers of organs to smaller numbers, Brefeld strongly supported the view that the basidium with its four spores has developed as a reduction process from a septate conidiophore which originally gave rise to an indefinite number of conidia. One of his lines of evolution runs from the smuts, with their "hemibasidia" and yeastlike budding of spores, through the rusts, Auriculariaceae and Tremellaceae with their "protobasidia," each of the four cells producing a single spore, to the autobasidiomycetes of the mushroom type. It is in the line of "Protobasidiomycetes" that Pilacre is brought forward in support of the main argument in favor of his theory, namely, that basidia and conidiophores, and basidiospores and conidia are, respectively, homologous.

According to Brefeld, the small, grayish sporocarps of Pilacre frequently found on old beech logs are the equivalent of the ordinary mushroom fruit body. In dissecting the fruiting heads he found that the spores were formed on true basidia. Each basidium had exactly four cells and each cell bore only one spore. Though recognizing the very dissimilar characters of the fruiting structure, he places Pilacre among the "Protobasidiomycetes" next to the Auriculariae, but differing from the Auriculariae in being angiocarpous and in having a gleba. The basidia were not developed in a definite hymenial layer.

In Brefeld's first cultures (*6*)[2] obtained by germinating spores from the fruiting heads only a hyphomycetous conidial form developed. In view of the fact that he could find no such conidial stage described and had not found it in nature he assumed that its production was due to the unnatural environmental conditions of his culture.

MATERIAL AND METHODS

Pilacre faginea has been collected by the senior author in localities as widely separated as New York and Florida. Although it occurs most frequently on beech logs it has been found on Acer, Carpinus, and other hardwoods, and typical specimens were found growing in the Florida everglades on wood of some subtropical tree.

The material for the cultures upon which the discussion in this paper is based was obtained in September, 1921, from specimens growing on a beech log near Chain Bridge, Va., a few miles from Washington.[3] Poured plates of corn-meal agar were made, using spores from the fruiting head. Sixteen single spore cultures were isolated and grown either at room temperature or in the refrigerator. Subcultures were grown on corn-meal

[1] Received for publication June 16, 1924—issued May, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 417.
[3] The writers are indebted to Angie M. Beckwith for assistance in the culture work.

agar, beech-decoction agar, and on sterilized beech branches. Large test tubes about 1 inch in diameter and 12 inches long proved to be most satisfactory for this work. As only one of the first cultures growing in small test tubes developed the basidial stage, other single-spore cultures were transferred to the large test tubes. These cultures were kept at room temperatures at first, but later some of them were placed in the refrigerator.

CHARACTERISTICS OF THE FUNGUS IN CULTURE

The basidiospores germinate in the manner described by Brefeld (*6*). The hyphae with their much branched conidiophores and luxuriant development of conidia have been so adequately and accurately described and figured by this author that further space need not be given to this phase of the question, except to call attention to certain characters not fully brought out by him. The mycelium in mass is not yellowish, as he describes. The production of conidia begins within a few days, and as soon as they cover the surface the color of the culture on potato-dextrose agar is Prout's brown, while on cornmeal agar it is Verona to sepia brown.[4] Grown on pieces of beech branches, the culture becomes covered with a great felty mass of mycelium and conidia, nearly Prout's brown in color. The mycelium grows very slowly; a colony several weeks old may not be over 2 cm. in diameter.

The fact that basidia, which are not easily recognized because of their form and manner of septation, are not developed in a hymenium and that their spores after becoming detached lie entangled among the loose hyphae of the head, has led some of our mycologists to a misconception as to the nature of the sporocarps. The hyphomycetous conidial stage which Brefeld first described and which always develops in cultures with the production of masses of conidia (pl. 2, A to C) comprises a phase in the life history entirely distinct from the basidial fructification.

After the first cultures had grown in 12-inch test tubes in the laboratory at room temperature for about four months, little flecks of pure white hyphae developed in several of the tubes. These growths increased in size, being at first rather indefinite in shape, but soon resembling very small mushroom buttons. The stipelike portion became surmounted by a small head. After two weeks some of the white growths began to turn grayish. These were removed and found to consist of hyphae having clamp connections. Basidia were developing from these hyphae in much the same way as figured by Brefeld. The basidiospores are nearly spherical, and being somewhat larger than the conidia they were easily distinguished from them. Brefeld has given adequate figures of the basidium and its spores. It is a true basidium having a definite number of spores, four in each case, just as claimed by him.

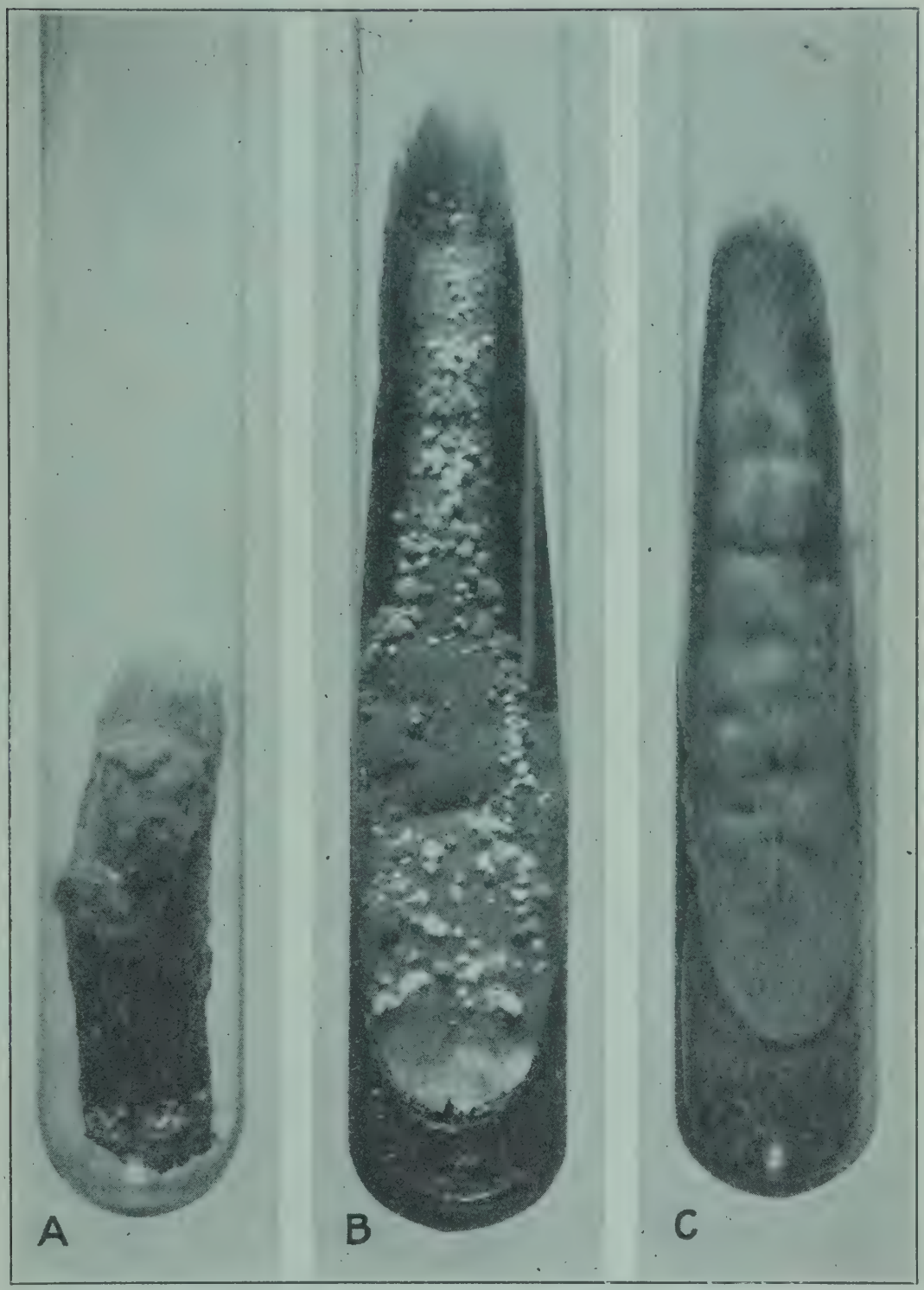
The first cultures, which were isolated November 5, 1923, fruited the following February, having meanwhile developed merely the gametophyte mycelium which produced only conidia. Sixteen monosporous cultures were transferred March 10; on June 5 it was found that most of the cultures showed the white patches of mycelium, which indicates that clamp connections are being formed and that basidia will soon develop. Ordinarily it requires about three months for the cultures to mature fruit bodies. One culture on potato-dextrose agar showed nearly 100 separate masses of white mycelium. Each mass represented an incipient fruit body covered with the ash-gray "gleba" in which quantities of basidiospores were being produced (pl. 1, B).

Cultures in which two monosporous strains in various combinations were grown did not produce the basidial fruiting stage in any greater abundance than did the single spore cultures. Fully as many fertile heads developed on cultures from one spore as were formed in cultures representing several strains.

No definite fruiting body such as characterizes Pilacre when it grows in nature on beech logs was developed in some of the cultures, although large numbers of basidia were formed in loose aggregations of hyphae. In other cases, however, fruiting bodies 2 or 3 mm. high were developed, some of them possessing a definite structure, consisting of a stipe surmounted by the expanded fertile head. The sporophytic hyphae of those which eventually produce basidia are pure white until the development of spores begins. The head then turns ash-gray as noted. Clamp connections are always found on the hyphae developing basidia. They also occur on the hyphae composing the so-called "peridium" (pl. 2, Q).

Wherever basidia are formed the hyphae producing them are clustered

[4] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C., 1912.

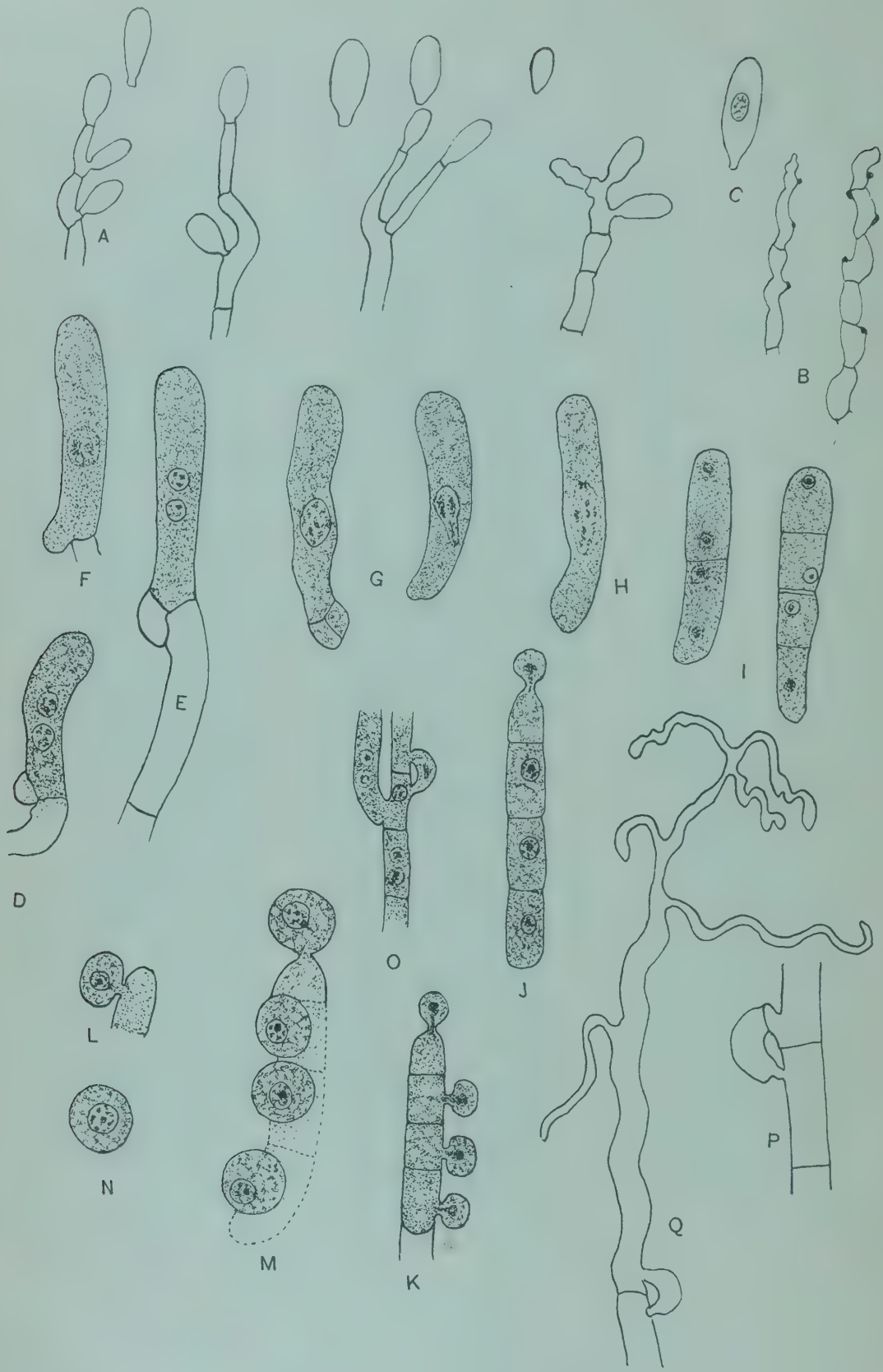


Cultures obtained by germinating basidiospores on potato dextrose agar, March 10, 1924. The cultures were photographed on June 9, natural size

A.—Single spore culture grown on sterilized beech twig showing only the conidial stage at the end of 3 months

B.—Parallel culture, also from a single spore; there was a large number of basidial fruit bodies developed on the surface of this culture

C.—Cultures which showed only the conidial stage after 3 months



The figures have the same magnification

A.—Ends of young conidiophores
B.—Rhinotrichum-like conidiophores
C.—Mature conidium
D to M.—Stages in the development of basidia and spores
N.—Mature spore
O to Q.—Cells of sporophytic hyphae showing clamp connections

together and not spread out indefinitely in the way one finds the mycelium producing basidia in Exobasidium or *Corticium vagum*, for example. A true peridium is not developed in Pilacre; the interlocking and entangling of the end branches of the sterile sporophytic hyphae make up an outer layer that has been called its peridium.

GAMETOPHYTIC AND SPOROPHYTIC GENERATIONS OF PILACRE

No fungus heretofore described illustrates more beautifully than does Pilacre the difference which may exist between the gametophytic and sporophytic stages in its life history. In this fungus the hyphae that produce conidia are gametophytic, those producing basidia are sporophytic. No conidia are developed on the latter kind of mycelium.

The gametophytic hyphae which arise from germinated conidia, basidiospores, or from bits of mycelium transferred to agar plates are of such a delicate structure and so narrow that it is difficult to differentiate cytoplasmic structures or to determine exactly the number of nuclei present in each cell. Some of the cells are extremely long and no doubt each cell then contains several nuclei. The conidia when first formed are uninucleated (pl. 2, C). Two masses of chromatin are sometimes visible in older spores, suggesting that the original nucleus may have divided. It is of interest to note that while no clamp connections can be found on the coarse brown hyphae-bearing conidia, these structures are easily found on the more delicate pure white hyphae which give rise to the basidia. Fixation of the sporophytic hyphae is rendered difficult by the quantity of air entangled in the hyphae of the "peridium." One occasionally finds cells with two nuclei (pl. 2, O). Sometimes cells are very long and these probably contain several nuclei. The fact that nuclei are sometimes found in pairs has no sexual significance. It is well known that when a fungus nucleus divides, the daughter nuclei tend to remain close together in pairs and only separate when they, in turn, divide. The branches that develop into basidia certainly arise at the clamp connection (pl. 2, D and E). The behavior of the nuclei at the time of clamp formation has not been followed, and an examination of additional material will be required in order to determine exactly what takes place at the origin of basidium. Presumably, the two nonsister nuclei which come together in a basidium clamp ordinarily fuse without further division. Brefeld (*6*) figures clusters of basidia in a way which suggests that there may be a great deal of proliferation at this time. It is well known that in the Ascomycetes a number of asci may arise as the result of such growth. The ultimate cell may also fuse with the antepenult, the resulting cell either becoming an ascus or by further growth and proliferation in turn give rise to another cluster of asci. Kniep's contention (*22*) that the clamps of Basidiomycetes and the crosiers of Ascomycetes are homologous structures would have been strengthened had he cited Brefeld's figures of Pilacre basidia as they arise from clamps. Such a suggestion implies that one accepts the theory that the Basidiomycetes and Ascomycetes have a common ancestral line. If so, there must be some very fundamental reason why the basidium becomes 4-nucleated and the ascus becomes 8-nucleated after the reduction divisions.

Such stages in the development of basidia as are shown in Plate 2, D to I, are abundant in sections of the sporocarps. It is clear that the cell wall in the middle of the basidium is laid down very early (pl. 2, I), even before the second division of the nucleus. The fusion nucleus, larger than either of the nuclei in the pair, is first seen. After a second division the nuclei are very small, each one containing a relatively large nucleolus. Figures obtained by the writers show that the basidium increases proportionately less in length than in diameter as it matures (pl. 2, J to M). It is not unusual to find one spore at the tip of the terminal cell (pl. 2, K). Not infrequently the sterigma is found at the side of the last cell of the basidium bringing all four spores into line (pl. 2, L). In Ascomycetes which have fruit bodies of any considerable size, by far the greater bulk of the tissue composing the ascocarp is gametophytic. The ascogenous hyphae proceeding from the ascogonium or from the cells which perform the function of an ascogonium are sporophytic in nature, and frequently compose in part what is called the subhymenium. In cleistocarpic ascocarps the tissue, sometimes erroneously called the peridium, which makes up the wall and overlies the hymenium, is gametophytic. The peridial hyphae of Pilacre are composed of the end branches of the hyphae, which are massed together below in parallel arrangement to form the stipe. Reaching the head, they develop the curiously contorted branches which, interlocking, form the peridium, through which the spores are slowly sifted out. As the fruit ages,

the peridial branches break away, exposing the spores tangled in the gleba. The peridial branches are first grayish in color and are scarcely to be distinguished from the rest of the young fruit body. As soon as spores are formed the head becomes ash-gray or cinereus. It is not difficult to determine by the change in color of the head when the first basidiospores are formed. As noted previously, the peridium is composed of sporophytic hyphae. This is proved by the fact that clamp connections regularly occur on these hyphae. In a culture bearing only conidia one looks in vain for such clamp connections, but as soon as the whitish flecks or mounds of mycelium develop on the conidial layer of the gametophytic mycelium, clamp connections can be found on those hyphae connected with the white mycelium above. The contrast between the coarse brown mycelium of the Rhizoctonia stage of *Corticium vagum* and the delicate whitish sporophytic hyphae bearing basidia is not greater than is found in the two stages in the life history of Pilacre.

Brefeld (*7*) drew his figures of hyphae bearing basidia and basidiospores from fruit bodies developing on bark. He germinated these spores and obtained cultures which produced only conidia. Later (*8, p. 164*) he reports producing the complete life cycle from basidiospores in culture. While these figures were drawn with the same care which he always gave to the details, when it comes to interpretation of his findings, he is moved to magnify similarities and to discount differences in comparing the basidia and their spores with the conidiophores and their conidia. As noted above, believing that a protobasidium is derived in the process of evolution from a conidiophore becoming septate or reduced to a definite structure with ultimately four cells, each cell producing a single spore, he says that the basidiospores and the conidia of Pilacre are about the same size, differ only a little in form, arise in exactly the same way, germinate exactly alike, and produce identical mycelia. In reality, the conidia and basidiospores are very different in color, they are absolutely dissimilar in form and are borne on hyphae with entirely different characteristics and origin. Of course the mycelium that develops from a germinated basidiospore could not differ from the mycelium which develops from a conidium, both structures being gametophytic. Hyphae giving rise to basidia are snow white, of delicate proportions, have clamp connections and sterile tip branches which take part in the formation of the "peridium." Clamp connections are now looked upon as an indication of the sporophytic condition of a mycelium. The recent work which has been done on the development of mushroom fruiting bodies from two strains of mycelium shows that in certain cases no clamp connections are formed in cultures from a single strain, but when two strains properly chosen are grown together so that anastomoses can occur, one begins to find clamp connections, and later the mushroom fruit body will develop. In such forms the binucleated condition of the hyphal cells is supposed to arise only along with or after clamp connections are developed.

The presence of clamp connections on hyphae is commonly accepted as an indication that the fungus belongs in the basidiomycete line, but failure to find such structures does not mean that the fungus must be excluded from this group. For example, Gilbert (*20*) did not find clamp connections on the hyphae of Dacryomyces. Here in some way, not determined, either as the result of anastomoses or by division of the nuclei, the cells below the hymenium become binucleated. After fusion of the two nuclei in the young basidium and the reduction divisions which follow, two of the four nuclei degenerate so that only two basidiospores are developed. Juel (*21*) as the result of cytological studies of *Stilbum vulgare* Tode believes that the fungus is a true Basidiomycete. He finds no clamp connections. On the other hand, the spores are regularly formed on club-shaped structures which originate as binucleated cells. The two nuclei then fuse. The fusion nucleus divides once and before the second division occurs a septum is laid down so that the basidium becomes 2-celled with one nucleus in each cell. The second division now occurs, but one of the daughter nuclei in each cell degenerates, so that only two basidiospores are developed. Juel points out that Pilacre and Pilacrella are angiocarpous while Stilbum is gymnocarpous. The peridium or covering surrounding the fertile tissue in the first two forms is of the simplest character.

According to Brefeld's figures, one can see that the conidia vary considerably in shape and size, some being long and narrow, others oval or nearly spherical. Some are exceedingly small, others very large. Lack of uniformity in shape and size is as striking as in case of the conidia of Cladosporium, for example. The conidial stage of Pilacre is to be looked upon as a separate and distinct spore form in the life history of

the fungus. Since conidia develop abundantly on agar cultures and on pieces of sterilized beech branches in test tubes, it is to be expected that this stage will be found in nature if one looks for it at the right time.

TAXONOMIC AND NOMENCLATORIAL RÉSUMÉ

This fungus, like most others, has had several generic and specific names applied to it. So far as known at present it was first described by Fries (*13*) in 1818 as *Onygena faginea*. Fries placed the fungus among the Gasteromycetes. This, however, is of little significance when we consider his conception of that group, which is indicated by the fact that he included in it not only true puff balls but also many Myxomycetes, Hyphomycetes, Discomycetes and even Agarics, for example, Nyctalis. The chief character upon which he relied, apparently, in determining relationship in these cases was the production of a powdery mass of spores of some kind.

There seems to be no doubt in regard to the fungus described by Fries as *Onygena faginea*, as the writers are informed by Professor Juel of Upsala, Sweden, who has kindly searched Fries's herbarium for them that there are several specimens of this species to be found there. One was sent by Schweinitz (*34, p. 65*) under the name *Onygena decorticata* Pers. and is mentioned by Fries (*16, p. 209*) as *O. faginea* Fr. and not Persoon's species (*29, p. 72*). The writers have examined duplicates of this gathering from Schweinitz's collection and find them to be typical *Pilacre faginea* (Fr.) B. and Br. There is also a specimen from Ohio in Fries's herbarium sent by Berkeley under the same name, and a Swedish specimen, which Professor Juel says "probably ought to be considered as the type of the species *Pilacre faginea*. It is labeled in Fries's hand "*Ecchyna faginea* Fr. Nobilissima Femsjo." Professor Juel says (in litt.) of this specimen "as far as I can see it is a typical specimen of what has been called "*Pilacre petersii* or *P. faginea* which I suppose to be synonymous."

It requires very little study of this fungus to show that it does not belong in the genus Onygena of Persoon, the type of which is the common discomycete *O. equina*, found on decaying hoofs and horns of animals. The generic name Onygena is now generally accepted and applied to the discomycete and probably no one would now suggest applying it to *Pilacre faginea*.

Fries's first description of the genus Pilacre was written in 1825 (*15, p. 364*). This description is too general and indefinite to connect it with any specific fungus at present. At the end of the description *Stilbum incarnatum* Weinmann (in litt.) is cited. Just what plant Fries had from Weinmann under this name no one has yet determined. Professor Juel has kindly searched through Fries's herbarium for the specimen of this from Weinmann but has been unable to find it under Pilacre or any of the various generic names which have at one time or another been suggested in connection with it; for example, Ecchyna, Onygena, Stilbum, and Roesleria. Professor Juel thinks it probable that the plant referred to was a lichen and the description certainly suggests Coniocybe or Calicium. He finds under Calicium, specimens from Weinmann but nothing labeled *incarnatum* or *Weinmannii*. Fries, in 1829 (*16, p. 204*), renamed the fungus, dedicating it to the collector, Doctor Weinmann of Petrograd. Dr. de Jaczewski of Petrograd has kindly made a search for Weinmann's collections. In a letter just received he says: "About the collections of Weinmann, I am sorry to say there is nothing left, neither at the Academy nor anywhere else." This species is therefore indeterminable and must be abandoned.

Boudier (*4*) discussed Pilacre and asserted that it is synonymous with Roesleria of Von Thümen. He based his conclusion primarily on the supposition that *P. weinmannii* Fr., the original species of the genus, is identical with *Roesleria hypogea* Thüm. There is nothing in the original description of Fries to justify such a conclusion and he gives no evidence of having seen the type or authentic specimens. It seems far more probable that the original specimen of Weinmann was a lichen, as already mentioned and suggested by Professor Juel. The original name *incarnatum* of Weinmann alone would seem to preclude the possibility of the fungus being Roesleria, which has no suggestion of such color in any stages of development. If further evidence were desired, it would be found in the statement that *incarnatum* was found growing on bark while *Roesleria hypogea* always, so far as known, grows underground on roots.

Fries's last mention of the genus was in 1849 (*17, p. 361*) where he gives a generic description only and says "Cfr. Weinm, Ross," referring to Weinmann's paper (*37*). Since Fries's monotype of the genus can not be determined and since no other species were ever referred

to it by him, it would seem that the name should be abandoned or a type for it sought elsewhere.

Weinmann (*37*) referred two more species to the genus Pilacre, *P. subterranea* and *P. friesii*. These species are now both regarded by some authors as being synonomous with the Discomycete *Roeslerea hypogea* Thüm. et Pass (see Beckwith, *2*). In 1834 Weinmann (*38*) transferred his *Pilacre friesii* of 1832 to the genus Onygena and described another species, which he called *Pilacre friesii* and which, according to the original description, might be regarded as a synonym of *Pilacre faginea*. All these species of Weinmann are somewhat doubtful, as no type or authentic specimens have been seen or identified by recent authors.

It is very clear from the description of this genus and the species referred to, that the authors had very little definite knowledge of the structure and relationships of the specimens with which they were dealing. It will be noted from the account given above of Fries's treatment of his genus Pilacre that he never referred to it his *Onygena faginea*. This was first referred to the genus by Berkeley and Broome in 1850 (*3, p. 365*). It is clear from Fries's treatment of *O. faginea* that he was more or less doubtful from the beginning as to its relationship, and finally in 1857 (*19, p. 151*) decided to call it *Ecchyna faginea*.

The history of the genus Ecchyna also show how unsatisfactory his ideas of the application and limits of some of the names he proposed were, especially when they related to the smaller fungi, a real knowledge of which required a more or less careful microscopic study. Fries proposed this name Ecchyna in 1819 (*14, p. 80*), giving the name only and adding "l. c." What the "l. c." refers to the writers have been unable to determine. The first work cited above on the page contains no mention of this name so far as they can discover, nor have they been able to find mention of it in any of the earlier works referred to by him in the publication cited.

The next mention of this name by Fries, in 1825 (*15, p. 151*), is in an observation under the description of Onygena of Persoon, in which he says he finds a notable fungus with hornlike branches but without heads, which he has called Ecchyna, but it may be a monstrous form of *O. faginea*. His next mention of this generic name is in 1849 (*17, p. 446*) in a footnote in Latin under *O. faginea*, which we translate as follows: "There exists intermediate between this and *Onygena faginea* and the next [Lasioderma] a fungus provisionally named Ecchyna." A brief discussion follows in which he says "it is without asci."

His next reference to this genus is in 1851 (*18, p. 135*) in which he says in Latin:

> I have proposed Ecchyna for *Onygena faginea* only as a subgenus of Onygena. It is very different from Pilacre to which my friend Berkeley refers it. It might be justifiable to propose *Onygena faginea* as an entirely distinct genus, but however others may feel it is impossible for me, trusting to the harmony of nature to attribute great weight to microscopical characters which are easily interpreted by morphological and physiological laws.

This incidentally throws interesting light upon Fries's mode of reasoning and interpreting nature. Fries's next mention of this species is in 1857 (*19, p. 151*) where he simply gives the name in a list of the species of Gasteromycetes occurring in central Sweden. Whether he was still in doubt as to the full generic rank of the name is not indicated. It is, therefore, difficult to say when, if ever, the name was really proposed as a genus by Fries. Notwithstanding this, Patouillard, 1900 (*28, p. 37*), takes up Ecchyna as a valid generic name citing Fries's footnote of 1849 as the place of publication.

If the generic name of this fungus is to be selected on the basis of priority of publication, there are at least two much older names than Ecchyna, the application of which is undoubted. The first of these is Phleogena Link, 1833 (*24, p. 396*). Link described the genus and then the monotype, *Phleogena faginea* and cites *Onygena faginea* Fries, as a synonym. This name appears to have been entirely neglected and is the oldest valid name known at present according to the priority rule.

In 1854 (*9, p. 47*) Corda, recognizing that *Onygena faginea* Fries, was very different from the true Onygena of Persoon, gave the species another generic name, Botryochaete. Corda apparently overlooked or disregarded the fact that Link had already proposed a generic name for the same species. Neither of these names ever came into general use.

Berkeley and Broome in 1850 (*3, p. 365*) first referred *Onygena faginea* of Fries to the genus Pilacre, and this combination has been generally used ever since. The same fungus was described by Berkeley and Curtis in 1859 (*3, v. 3, p. 362*) from American specimens collected by Peters in Alabama and named *Pilacre petersii*. Saccardo in his Sylloge (*32, p. 362*) has referred ten species to Pilacre. Of these *P. friesii* Weinmann and *P. petersii*, B. & C. are apparently both synonyms of *P. faginea*, the latter certainly so. The other species mentioned are unknown to the writers; they can not

therefore say whether these are congeneric or not. *P. pallida*, E. & E. (*12, p. 59*) is a very different fungus, as the writers have determined by an examination of some of the original material upon which the species was based, and is certainly not congeneric with *P. faginea*.

In view of the fact as already indicated, that the original type of the genus Pilacre of Fries is unknown and that the name has been generally applied for the last 74 years to the fungus originally named *Onygena faginea* by Fries the writers propose to adopt Pilacre on the basis of general usage and assign to it as its type the common and well known species *P. faginea*, with which it has been so long associated. This procedure, they believe, will best serve the primary purposes and aims of binomial nomenclature, by fixing once for all with certainty the application of this well-known and established name, thus avoiding the necessity for any change in the future. It may be of interest to note that Möller (*26, p. 54*) arrived at the same conclusion in regard to the application of this generic name.

The synonomy of this species so far as known at present is as follows:

Onygena faginea Fr. 1818 (*13, p. 25.*)
Onygena decorticata Schw. 1822 (*34, p. 65.*) (Not *O. decorticata* Pers. 1799. *29.*)
Phleogena faginea (Fr.) Link 1833 (*24, p. 396.*)
Pilacre friesii Weinm. in Linnaea 1834 (*38, p. 413–414.*) (Not *P. fricsii* Weinm., in Flora, 1832. *37, p. 458.*)
Pilacre faginea (Fr.) Berk. & Br. 1850 (*3, p. 365.*)
Botryochaete faginea (Fr.) Corda 1854. (*9, p. 46.*)
Ecchyna faginea Fr. 1857 (*19, p. 151.*)
Pilacre petersii Berk. & Curt. 1859 (*3, p. 362.*)

Stilbum pilacreforme Rich. 1881 (*30*) and 1882 (*31, p. 241*), cited as a synonym by Beckwith (*2*), does not appear, according to Richon's description, to be Pilacre. However, the writers have not seen Richon's specimens and so can not speak positively.

MORPHOLOGY AND RELATIONSHIP

As already stated, Fries (*13*) referred this fungus to the Gasteromycetes. This, however, has little significance on account of his lack of knowledge of the morphology and development of the fungus and also as it was the result of the most superficial observation. Neither Link nor Corda, both of whom renamed the fungus, added any thing of value to our knowledge of it. Corda (*9, p. 46*), however, did consider it a basidiomycete and placed it next to the hypogaeous Gasteromycete, Melanogaster. He says that the family to which it belongs is doubtful and the genus is of uncertain affinity, but emphasizes the fact that it has basidia although he stated that they are 1- to 4-spored.

The next writer to consider the relationship of this fungus was Tulasne (*35, p. 294–295*). He first regarded Pilacre as related to Ptychogaster, the conidial or chlamydospore condition of a Polyporus. Later (*36, p. 228*) he investigated the spore formation of Pilacre and made another interpretation comparing it to *Hypochnus purpureus* and regarding it as related to the Auriculariae. De Bary (*1, p. 335*) does not seem to have made any particular study of this fungus and gives Tulasne's earlier interpretation of it. The next contribution to our knowledge of this plant was made by Schroeter in 1877 (*33*). In connection with a discussion of the systematic position of Tulostoma, he mentions Tulasne's studies of Pilacre and states that according to his illustrations of the spore formation, these two fungi are very similar, and he says that instead of being closely related to Hypochnus, he regards it as much more closely related to Tulostoma, and concludes with the statement: "I regard it as apparent that this fungus, Pilacre, which has heretofore been placed in different families, is the type as already noted of a second genus of the family Tulostomaceae."

It remained for Brefeld to give us the first clear and accurate account of the life history of this fungus. He grew it in culture and obtained a conidial condition which is a Hyphomycete similar in general structure and appearance to Rhinotrichum or Haplaria. He also gave detailed illustrations of its perfect condition with the typical transversely divided four-celled basidia and basidiospores. He regarded the fungus as closely related to the Auriculariales at first, but in his later summary or review of his work he mentions the similarity between the basidia of Pilacre and Tulostoma (*7, p. 197*) and also mentions the general resemblance of the fruiting bodies, both being angiocarpous; in his final arrangement of the fungi he places Pilacre intermediate between the Auriculariaceae and Lycoperdaceae. His idea in regard to the relation between the conidial form and the perfect stage and its significance as to the development of the Basidiomycetes from the Hyphomycetes is, of course, now generally regarded as untenable.

Möller (*26, p. 48–64*), one of Brefeld's students, was the next to treat of this fungus and some of its relatives. He found in Brazil a fungus which he re-

garded as identical with *Pilacre petersii* of Europe. He was, however, unable to germinate the spores of the fungus and so did not obtain pure cultures. He found, however, other fungi which he regarded as belonging to the Protobasidiomycetes and related to Pilacre. One especially interesting belongs to the genus Pilacrella, differing from Pilacre in having a much looser head without a distinctive peridium, but producing prolonged branching hyphae all about the head. This fungus also produces a conidial form of hyphomycetous character entirely distinct from the basidiomycetous fructification. The conidia are sometimes borne laterally on the four terminal cells of the hyphae, suggesting somewhat in appearance the basidia. This led Möller to consider this fungus as showing a perfect transition between the Hyphomycetes and the Basidiomycetes. Möller regarded Pilacre as being most closely related to Stypinella, a genus of Auriculariaceae. No recent additions have been made to our knowledge of Pilacre.

Lindau (*23, p. 86*) places the genus Pilacre in the Auriculariaceae. Saccardo (*32, p. 579*) places it in the Stilbaceae of the Hypomycetes, but speaks of its possible relation to Roesleria and its lack of a typical hyphomycetous character. He includes 10 species, most of which have been described by Berkeley and Broome and Berkeley and Curtis. These are all from the Tropics and very little is known in regard to their structure or relationship.

Bayliss-Elliott and Grove (*11*) also discussed the fungus and regarded it as probably the conidial condition of Roesleria. Overholts, 1922 (*27, p. 165*), says he was also able to find the cross walls in the basidia, which are characteristic of the Auriculariales; but says that the fungus departs widely in consistency and habit from other members of the order. Lloyd (*25*) in a recent reference to the fungus considers it a hyphomycete.

Beckwith (*2*) has pointed out that many authors have confused Pilacre with Roesleria and Coniocybe, some claiming that *Pilacre petersii* is merely a conidial stage of Roesleria. She has shown by pure cultures that Roesleria develops only ascocarps in culture.

As a result of the writers' studies of this fungus and those cited bearing on this and other fungi which are more or less related to it, they are of the opinion that it represents what might be appropriately called a protogasteromycete. Its nearest known relative at present among the Gasteromycetes is apparently Tulostoma, as suggested by Schroeter (*33*). It is not unlikely that when the fungi of the world are more fully known other genera will be found partially bridging the gap between genera like Pilacre and Tulostoma.

As already indicated, Brefeld and Möller showed that *Pilacre faginea* possesses true basidia, and on account of these being transversely septate and bearing lateral spores regarded it as more closely related to the Auriculariales than to the Gasteromycetes. Brefeld (*5, p. 194*) at first, basing his opinion on Tulasne's account of Pilacre, stated that the Gasteromycetes had most probably arisen from a similar form. Schroeter pointed out its angiocarpous character and the resemblance of the basidia and spore formation to that of Tulostoma.

Eidam (*10*) has shown the presence of clamp connections in Cyathus and Crucibulum, while so far as the writers know at present, these have not been found in the Auriculariales. This would also appear to indicate the closer relationship of Pilacre to the Gasteromycetes than to the Auriculariales.

SUMMARY

The life history of *Pilacre faginea* as determined by Brefeld has been verified by pure cultures from basidiospores and also from conidia, the perfect or basidiomycetous stage being produced in both cases. It is shown that this fungus has true basidia, each arising from a clamp connection, which is a two-nucleate structure. These nuclei fuse and undergo two divisions so that the mature basidium consists of four uninucleate cells.

The fungus is shown to possess two distinct stages of development, the gametophytic and sporophytic, differing in the color and character of the mycelium and in the character and method of production of the spores. The conidial stage (gametophytic) has the general appearance of a hyphomycete similar to Rhinotrichum or Haplaria. The basidial stage (sporophytic) has the general characteristics of a Basidiomycete, having clamp connections and uniformly four-spored basidia. The sporophytic stage produces a distinct more or less stipitate sporocarp having a gleba and peridium suggestive of a Gasteromycete. Brefeld and Möller, who made careful studies of the fungus, regarded it as intermediate between the Auriculariales and Gasteromycetes. Schroeter regarded it as having closer affinities with the Gasteromycetes.

The evidence accumulated by the writers leads them to regard this fungus

as a Protogasteromycete, whose nearest known relative among the puff balls appears to be Tulostoma.

The history of the nomenclature of this fungus shows a difference of opinion as to the application of the generic name Pilacre. There seems to be no good reason for regarding it as a synonym of Roesleria, as the original type of the genus is unknown and undetermined.

The writers adopt and recommend the application of the generic name Pilacre to *P. faginea* as the nomenclatorial type of the genus because this name has been established and in general use for this fungus for the past 74 years.

It is shown that this fungus has no relation to Roesleria, the Discomycete which some authors have considered as a perfect stage of *Pilacre faginea*.

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TOXICITY STUDIES WITH DICYANODIAMIDE ON PLANTS[1]

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INTRODUCTION

The conclusions reached from the experimental work dealing with the effect of dicyanodiamide on plant growth which have been reported by various investigators vary widely. In some instances the compound has been reported as very toxic to plants, in others as merely unavailable as a nitrogen source for plants, and practically inert. At the Fixed Nitrogen Research Laboratory of the United States Department of Agriculture as well as elsewhere, experiments have shown that small quantities of dicyanodiamide prevent nitrification in the soil. It seemed, therefore, that possibly the bad effects of the compound might be due to the fact that in its presence the soil nitrogen is not converted into nitrates or at least is converted so slowly that plants undergo nitrate starvation. Thus dicyanodiamide would act as an indirect rather than direct poison to plants. The experiments reported here were planned to determine this point by studying the growth of plants in soils containing dicyanodiamide and varying quantities of sodium nitrate.

SOILS AND PLANTS USED IN THE EXPERIMENTS

The soils used were Norfolk sandy loam, a Coastal Plain soil obtained from near Norfolk, Va., and Chester loam, a Piedmont Plateau soil secured from near McLean, Va. The plants used were wheat and cowpeas.

SOIL PREPARATION AND FERTILIZATION

The materials to be tested were thoroughly mixed with each 10-pound portion of sieved soil and put into 1-gallon glazed pots and planted, each treatment being in duplicate. After germination was complete the plants were thinned to 10 and 5, respectively, for wheat and cowpeas.

All pots except those designated as "no fertilizer" received 80 pounds of P_2O_5 and 40 pounds of K_2O per acre in the form of monocalcium phosphate and potassium sulphate, respectively. The calculations were based on 2,000,000 pounds of soil per acre to a depth of 6 inches. The sodium nitrate and dicyanodiamide were applied at various rates and in varying proportions. Check pots were included in which the normal applications of phosphate and potash were made, but no nitrogen. In the tables which follow, the term fertilizer ratio refers to the ratio of NH_3, P_2O_5, and K_2O in the order named.

DURATION OF EXPERIMENTS

The wheat was planted on March 18, 1922, and cut on May 1, 1922. During this period the pots were weighed at frequent intervals and the water lost by evaporation restored. The wheat was harvested sooner than desired because of an unusually severe attack of mildew.

The cowpeas were planted on May 16, 1922, and cut on June 21, 1922. The excessive heat in the greenhouse was causing many of the lower leaves to drop, making necessary the early termination of the experiment.

EXPERIMENTAL RESULTS

First Experiment

RESULTS WITH WHEAT.

A. Sodium Nitrate Constant, Dicyanodiamide Variable

The results from pots of wheat treated with varying quantities of dicyanodiamide, together with a constant and adequate nitrate supply, are given in Table I and Figure 1. Tests were also included using sodium nitrate and dicyanodiamide singly for comparison.

[1] Received for publication June 5, 1924; issued May, 1925. The experiments herein reported were conducted at Arlington, Va., the Soil Fertility greenhouses being used.

These figures show that dicyanodiamide at the high rate of 40 pounds of ammonia per acre is only slightly toxic to wheat under the conditions used. On the sandy Norfolk soil the decrease in weight of the plants amounted to approximately 17 per cent and on the Chester loam to 13 per cent, as compared with the control. Where sodium nitrate was used together with different quantities of dicyanodiamide, the green weights were practically as large with the dicyanodiamide as without in the case of the lighter soil. The dicyanodiamide did cause a slight drop at the highest rate, but this was almost within experimental error. On the Chester loam the results were similar, except that both 20 and 40 pounds of ammonia as dicyanodiamide used with sodium nitrate caused slight decreases as compared with nitrate alone. These results seem to show that dicyanodiamide is toxic to wheat to a slight extent at the high rate used but can not be considered as a marked direct poison. It did not greatly lessen the fertilizing value of sodium nitrate or greatly affect the growth of the plants.

The observations made during growth of the wheat corresponded rather closely to the final green weights. Dicyanodiamide caused a slight tip-burning at all rates of application on the Norfolk sandy loam, the burning being greatest when the plants were about 2 weeks old. There was a partial recovery later. At the rates of 20 and 40 pounds of ammonia, many of the leaf tips were burned back 2 to 3 inches. The rate of growth did not appear to be greatly affected, however. The burning effect was not lessened by application of sodium nitrate. On the Chester loam there was practically no burning at any time.

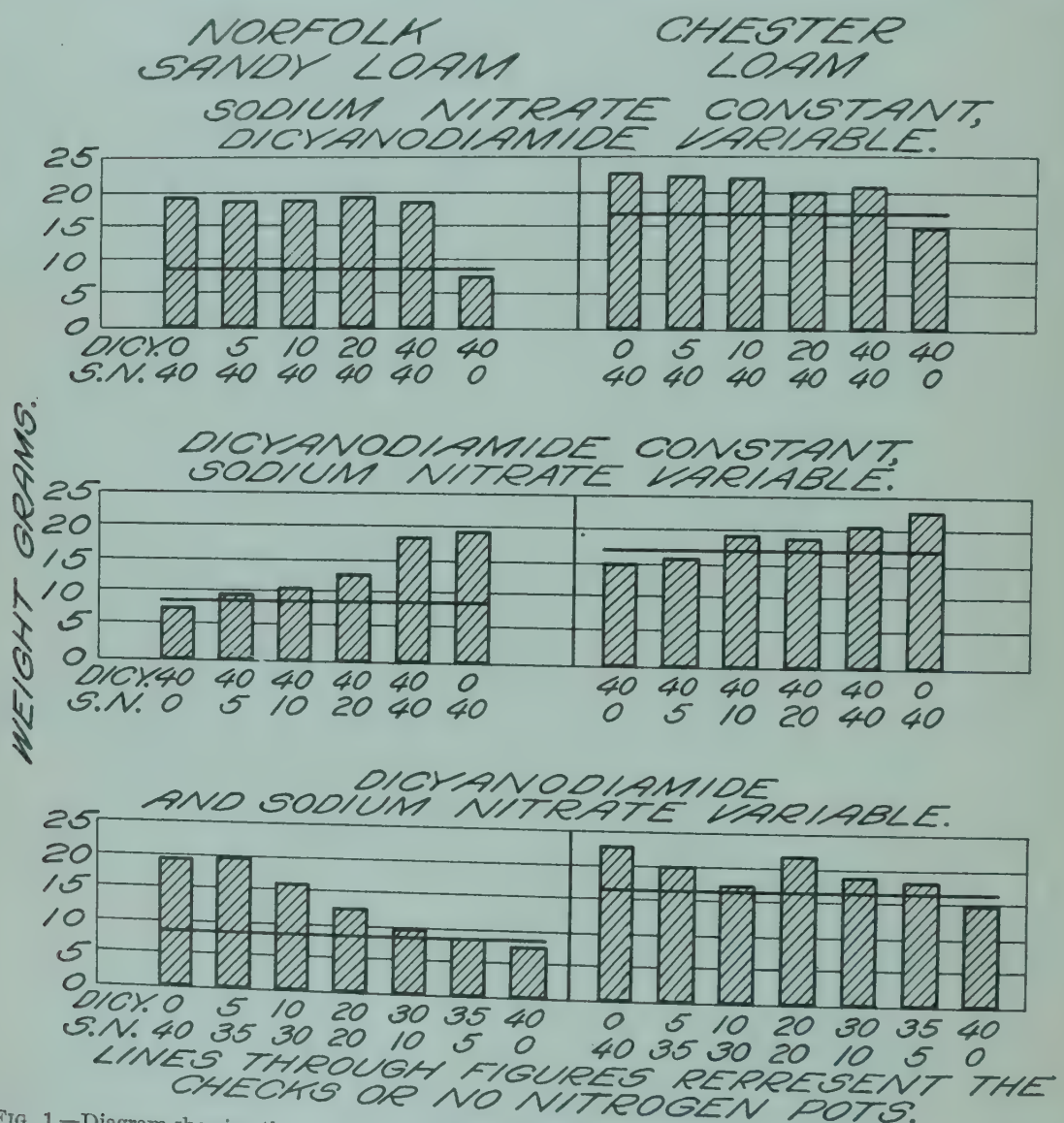

NORFOLK SANDY LOAM
CHESTER LOAM
SODIUM NITRATE CONSTANT, DICYANODIAMIDE VARIABLE.
DICY. 0 5 10 20 40 40
S.N. 40 40 40 40 40 0
0 5 10 20 40 40
40 40 40 40 40 0
DICYANODIAMIDE CONSTANT, SODIUM NITRATE VARIABLE.
DICY. 40 40 40 40 40 0
S.N. 0 5 10 20 40 40
40 40 40 40 40 0
0 5 10 20 40 40
DICYANODIAMIDE AND SODIUM NITRATE VARIABLE.
DICY. 0 5 10 20 30 35 40
S.N. 40 35 30 20 10 5 0
0 5 10 20 30 35 40
40 35 30 20 10 5 0
WEIGHT GRAMS.
0 5 10 15 20 25
LINES THROUGH FIGURES REPRESENT THE CHECKS OR NO NITROGEN POTS.

FIG. 1.—Diagram showing the yields of wheat from pots receiving various amounts of dicyanodiamide and sodium nitrate

TABLE I.—*Green weights of wheat from pots receiving various quantities of dicyanodiamide in the presence of a constant nitrate supply*

Treatment [a]	Fertilizer ratio NH_3-P_2O_5-K_2O	Norfolk sandy loam			Chester loam		
		Green weight	Average	Increase or decrease over check	Green weight	Average	Increase or decrease over check
		Grams			*Grams*		
No fertilizer	0-0-0	8.4 8.0	8.2		12.7 12.6	12.7	
No nitrogen	0-8-4	9.2 9.0 9.3 8.0 9.2 8.5	8.9		16.3 15.8 14.4 17.9 22.1 13.9	16.7	
Sodium nitrate 40	4-8-4	18.2 17.8 18.9 21.7	19.2	10.3	22.4 21.4 23.0 24.2	22.8	6.1
Dicyanodiamide 40	4-8-4	6.8 7.9	7.4	−1.5	14.9 14.3	14.6	−2.1
Sodium nitrate 40, dicyanodiamide 5	4.5-8-4	18.8 18.9	18.9	10.0	22.8 21.5	22.2	5.5
Sodium nitrate 40, dicyanodiamide 10	5-8-4	18.4 19.4	18.9	10.0	21.2 22.8	22.0	5.3
Sodium nitrate 40, dicyanodiamide 20	6-8-4	20.4 18.0	19.2	10.3	21.5 18.4	20.0	3.3
Sodium nitrate 40, dicyanodiamide 40	8-8-4	17.6 19.0	18.3	9.4	20.6 20.4	20.5	3.8

[a] In this table and those which follow the figures refer to pounds of nitrogen per acre expressed as NH_3 (2,000,000 pounds of soil).

TABLE II.—*Green weights of wheat from pots receiving various quantities of sodium nitrate in the presence of dicyanodiamide*

Treatment	Fertilizer ratio NH_3-P_2O_5-K_2O	Norfolk sandy loam			Chester loam		
		Green weight	Average	Increase or decrease over check	Green weight	Average	Increase or decrease over check
		Grams			*Grams*		
No fertilizer	0-0-0		8.2			12.7	
No nitrogen	0-8-4		8.9			16.7	
Sodium nitrate 40	4-8-4		19.2	10.3		22.8	6.1
Dicyanodiamide 40	4-8-4	6.8 7.9	7.4	−1.5	14.9 14.3	14.6	−2.1
Dicyanodiamide 40, sodium nitrate 5	4.5-8-4	10.2 8.9	9.6	.7	16.0 15.7	15.9	−.8
Dicyanodiamide 40, sodium nitrate 10	5-8-4	9.5 10.9	10.2	1.3	18.9 19.1	19.0	2.3
Dicyanodiamide 40, sodium nitrate 20	6-8-4	11.6 13.6	12.6	3.7	18.7 18.7	18.7	2.0
Dicyanodiamide 40, sodium nitrate 40	8-8-4	17.6 19.0	18.3	9.4	20.6 20.4	20.5	3.8

B. Dicyanodiamide Constant; Sodium Nitrate Variable

The green weights of wheat from pots receiving dicyanodiamide at a rate equivalent to 40 pounds of ammonia per acre with 0 to 40 pounds of sodium nitrate are given in Table II and in Figure 1. The data show that in the presence of sodium nitrate dicyanodiamide continues to exert an injurious, although not very noticeable, effect on the plants. Considering the pots receiving dicyanodiamide alone

as the check, it will be observed that sodium nitrate produced an increase of growth in about the same proportion as would be expected where the only source of nitrogen was nitrate. On the sandy loam nitrate equivalent to 5 pounds of ammonia per acre was more than sufficient to counteract the 40 pounds of ammonia as dicyanodiamide. On the Chester loam this quantity was not quite sufficient, but 10 pounds was more than adequate.

C. Dicyanodiamide and Sodium Nitrate in Varying Proportions; Nitrogen Constant

This experiment was planned so as to keep the nitrogen content constant throughout while supplying the nitrogen as dicyanodiamide and sodium nitrate in different proportions. Such mixtures would approximate ordinary fertilizer practice on the basis of nitrogen content, but would contain a portion of the nitrogen as unavailable dicyanodiamide. The data given in Table III and shown in Figure 1 confirm the results reported in previous tables, again demonstrating that dicyanodiamide is not markedly toxic and that the reduction in green weight is overcome by the application of very small quantities of sodium nitrate. The yields obtained with mixtures of the two materials depended almost wholly on the nitrate content, particularly on the sandy soil. The yields on the Chester loam were somewhat irregular and not always in agreement with those to be expected from the fertilizer treatments.

RESULTS WITH COWPEAS

In order to determine the residual effects of dicyanodiamide applications, a few of the pots which had grown a crop of wheat were planted to cowpeas without additional treatment. The green weights of the plants are given in Table IV.

The results obtained on the Norfolk sandy loam show a marked injury by dicyanodiamide even to the second crop, in fact greater than to the first crop. However, there seems to be no doubt that the cowpea is much less tolerant of dicyanodiamide than is wheat. This point is well illustrated in subsequent tables. During growth the cowpeas showed the same leaf yellowing and mosaic appearance as where fresh applications of dicyanodiamide had been used, but possibly to a slighter degree. This is in agreement with the results reported by the majority of investigators which show that dicyanodiamide is not readily decomposed in the soil. Under greenhouse conditions, where there was no leaching, the dicyanodiamide would necessarily have to be broken up chemically in order for its injurious effects to be eliminated, unless perhaps a small amount might be removed from the soil solution by absorption.

On the Chester loam the results obtained with cowpeas were somewhat indefinite, the mixture of dicyanodiamide and sodium nitrate giving slightly smaller green weights than the check, whereas both dicyanodiamide alone and sodium nitrate alone gave increases over the no-nitrogen treatment. The differences are not very significant.

TABLE III.—*Green weights of wheat from pots receiving varying quantities of both dicyanodiamide and sodium nitrate*

Treatment	Fertilizer ratio NH_3-P_2O_5-K_2O	Green weight	Average	Increase or decrease over check	Green weight	Average	Increase or decrease over check
		Grams			*Grams*		
No fertilizer	0-0-0	---------	8.2	---------	---------	12.7	---------
No nitrogen	0-8-4	---------	8.9	---------	---------	16.7	---------
Sodium nitrate 40	4-8-4	---------	19.2	10.3	---------	22.8	6.1
Sodium nitrate 35, dicyanodiamide 5	4-8-4	20.3 19.3	19.8	10.9	21.4 18.6	20.0	3.3
Sodium nitrate 30, dicyanodiamide 10	4-8-4	16.4 15.5	16.0	7.1	18.0 16.0	17.0	.3
Sodium nitrate 20, dicyanodiamide 20	4-8-4	12.2 12.3	12.3	3.4	21.5 22.1	21.8	5.1
Sodium nitrate 10, dicyanodiamide 30	4-8-4	8.3 10.8	9.6	.7	17.6 20.2	18.9	2.2
Sodium nitrate 5, dicyanodiamide 35	4-8-4	7.8 9.2	8.5	−.4	19.1 17.1	18.1	1.4
Dicyanodiamide 40	4-8-4	6.8 7.9	7.4	−1.5	14.9 14.3	14.6	−2.1

TABLE IV.—*Green weight of cowpeas grown after wheat without additional fertilization*

NORFOLK SANDY LOAM

Source of nitrogen •	Fertilizer ratio	Green weight	Average	Increase or decrease over check	Nodule formation
		Grams			
No nitrogen	0-8-4	26.0 24.3	25.2	----------	Abundant Do.
Sodium nitrate 40	4-8-4	25.4 25.4	25.4	0.2	Do. Do.
Dicyanodiamide 40	4-8-4	20.6 15.3	18.0	−7.2	Many. Do.
Dicyanodiamide 40, sodium nitrate 40	8-8-4	18.3 16.6	17.5	−7.7	Do. Abundant.

CHESTER LOAM

No nitrogen	0-8-4	35.6 35.1	35.4	----------	Few. Do.
Sodium nitrate 40	4-8-4	40.3 39.9	40.1	4.7	Many. Few.
Dicyanodiamide 40	4-8-4	38.7 36.0	37.4	2.0	Do. None.
Dicyanodiamide 40, sodium nitrate 40	8-8-4	31.2 34.1	32.7	−2.7	Few. Do.

• The figures in this column refer to the quantities of nitrogen applied to the crop of wheat, previously grown in this soil.

In Table IV, as well as in those which follow, a column is included showing the relative abundance of nodules. No doubt the abundance of nodules on the plants in some pots, especially in those containing the Norfolk soil, was quite a factor in determining growth.

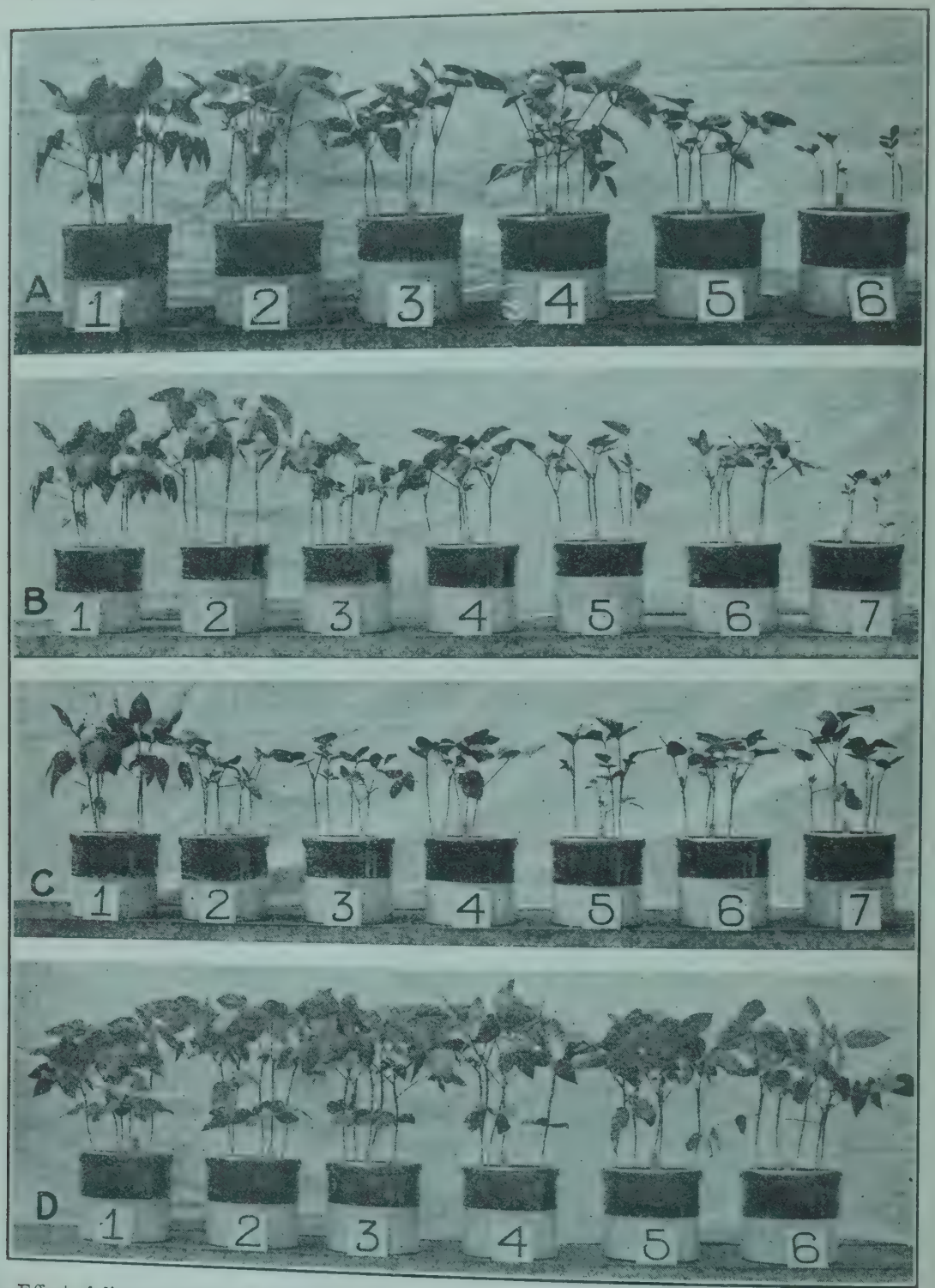
SECOND EXPERIMENT

EFFECT OF VARIOUS AMOUNTS OF DICYANODIAMIDE WITH AND WITHOUT SODIUM NITRATE ON COWPEAS

At the same time that the experiments with cowpeas reported on above were begun, another series was also included to determine the toxicity of dicyanodiamide to cowpeas and the relation of the nitrate supply to the injury. The results are given in Tables V and VI and illustrated in Figures 2 and 3. Plates 1 and 2 show the plants just prior to harvesting.

The results reported in Table V with the Norfolk sandy loam were somewhat unexpected. The soil which grew such a poor crop of wheat in the absence of a nitrogenous fertilizer showed negligible responses to sodium nitrate with cowpeas. Furthermore, small applications of dicyanodiamide depressed the yield quite markedly, while the larger quantities injured germination and practically killed the plants which did appear above the surface. In these extreme cases, the roots became brown and rotted. The use of sodium nitrate with the dicyanodiamide increased the injury up to 20 pounds of ammonia per acre and decreased it slightly thereafter. These results are decidedly different from those obtained with wheat.

The data for the Chester loam show a fair increase in green weight of the plants resulting from the use of sodium nitrate alone. Dicyanodiamide depressed the yields at 20 pounds of ammonia per acre and above. Where sodium nitrate was used with the dicyanodiamide the injury by the latter was partially overcome. The results obtained with cowpeas on this soil agreed more nearly with those obtained with wheat than was true with the lighter soil experiments. Nevertheless, the dicyanodiamide injury was greater for cowpeas than for wheat, as shown both by green weights and the yellowing and mosaic effects on the leaves.

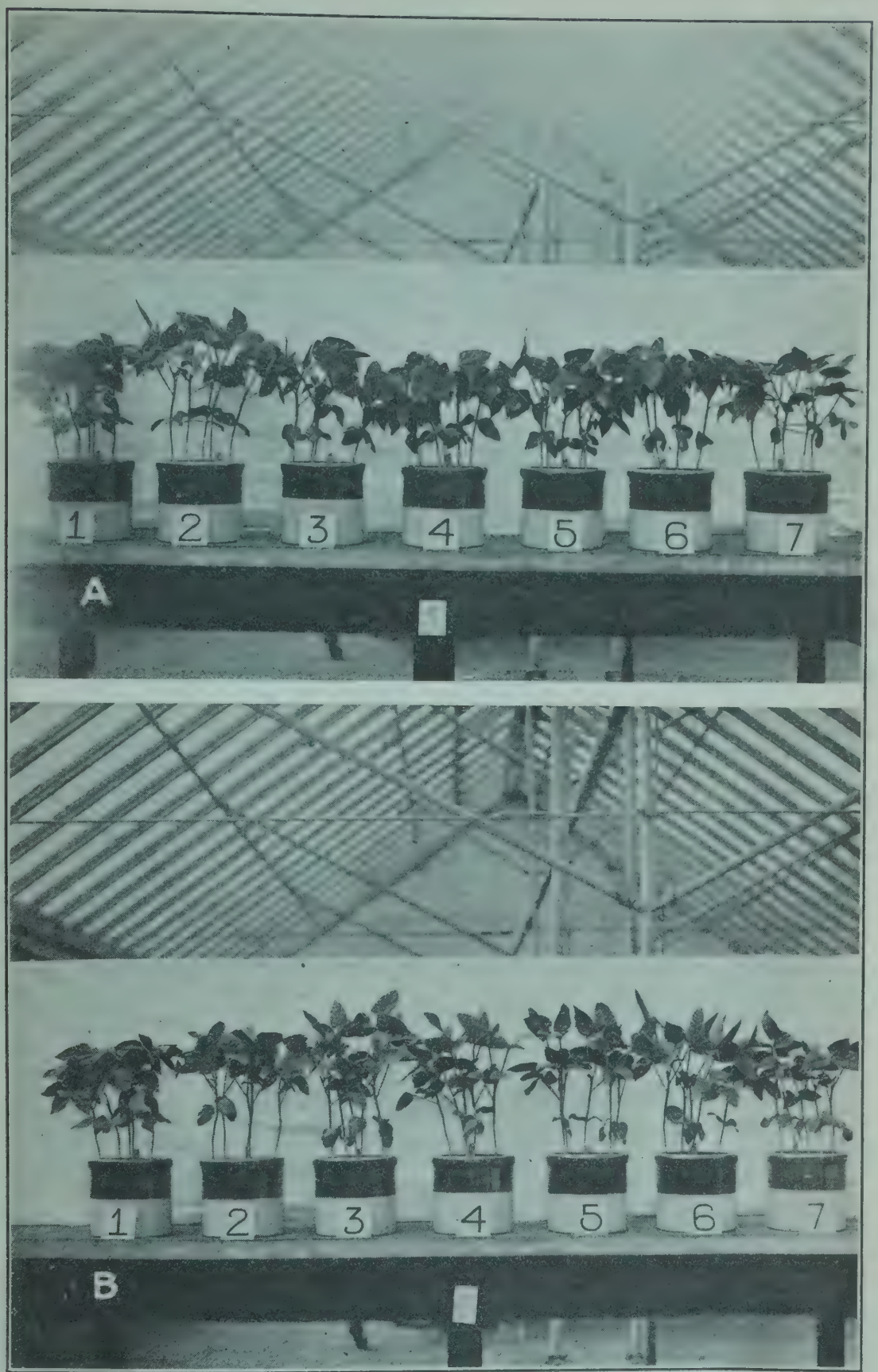


A.—Effect of dicyanodiamide on cowpeas grown on Norfolk sandy loam. The applications are expressed as pounds NH_3 per acre. 1, no nitrogen; 2, dicyanodiamide, 5; 3, dicyanodiamide 10; 4, dicyanodiamide 20; 5, dicyanodiamide 40; 6, dicyanodiamide, 80.

B.—Growth of cowpeas on Norfolk sandy loam, receiving a constant quantity of sodium nitrate and varying quantities of dicyanodiamide. The applications are expressed in pounds NH_3 per acre. 1, no nitrogen; 2, sodium nitrate 40; 3, sodium nitrate 40, dicyanodiamide 5; 4, sodium nitrate 40, dicyanodiamide 10; 5, sodium nitrate 40, dicyanodiamide 20; 6 sodium nitrate 40, dicyanodiamide 40; 7, sodium nitrate 40, dicyanodiamide 80.

C.—Growth of cowpeas on Norfolk sandy loam receiving a constant quantity of dicyanodiamide and varying quantities of sodium nitrate. The applications are expressed as pounds NH_3 per acre. 1, no nitrogen; 2, dicyanodiamide 40; 3, dicyanodiamide 40, sodium nitrate 5; 4, dicyanodiamide 40, sodium nitrate 10; 5, dicyanodiamide 40, sodium nitrate 20; 6, dicyanodiamide 40, sodium nitrate 40; 7, dicyanodiamide 40, sodium nitrate 80.

D.—Effect of dicyanodiamide on cowpeas grown on Chester loam. The applications are expressed as pounds NH_3 per acre. 1, no nitrogen; 2, dicyanodiamide 5; 3, dicyanodiamide 10; 4, dicyanodiamide 20; 5, dicyanodiamide 40; 6, dicyanodiamide 80.



A.—Growth of cowpeas on Chester loam receiving a constant quantity of sodium nitrate and varying quantities of dicyanodiamide. The applications are expressed as pounds NH_3 per acre. 1, no nitrogen; 2, sodium nitrate 40; 3, sodium nitrate 40, dicyanodiamide 5; 4, sodium nitrate 40, dicyanodiamide 10; 5, sodium nitrate 40, dicyanodiamide 20; 6, sodium nitrate 40, dicyanodiamide 40; 7, sodium nitrate 40, dicyanodiamide 80

B.—Growth of cowpeas on Chester loam receiving a constant quantity of dicyanodiamide and varying quantities of sodium nitrate. The applications are expressed as pounds NH_3 per acre. 1, no nitrogen; 2, dicyanodiamide 40; 3, dicyanodiamide 40, sodium nitrate 5; 4, dicyanodiamide 40, sodium nitrate 10; 5, dicyanodiamide 40, sodium nitrate 20; 6, dicyanodiamide 40, sodium nitrate 40; 7, dicyanodiamide 40, sodium nitrate 80

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EFFECT OF VARIOUS QUANTITIES OF SODIUM NITRATE WITH AND WITHOUT DICYANODIAMIDE ON COWPEAS

The data given in Table VI for the Norfolk sandy loam further emphasize the results reported in Table V. Applications of sodium nitrate had little effect on the growth of cowpeas either when used alone or with dicyanodiamide. In the latter case the nitrate usually increased the injury slightly instead of counteracting it.

The relative abundance of nodules on the roots of the cowpeas was not appreciably affected by the treatments, except that quantities of dicyanodiamide large enough to injure plant growth usually inhibited nodule formation. The untreated pots were usually the best inoculated.

SUMMARY

In pot experiments using Norfolk sandy loam and Chester loam, the

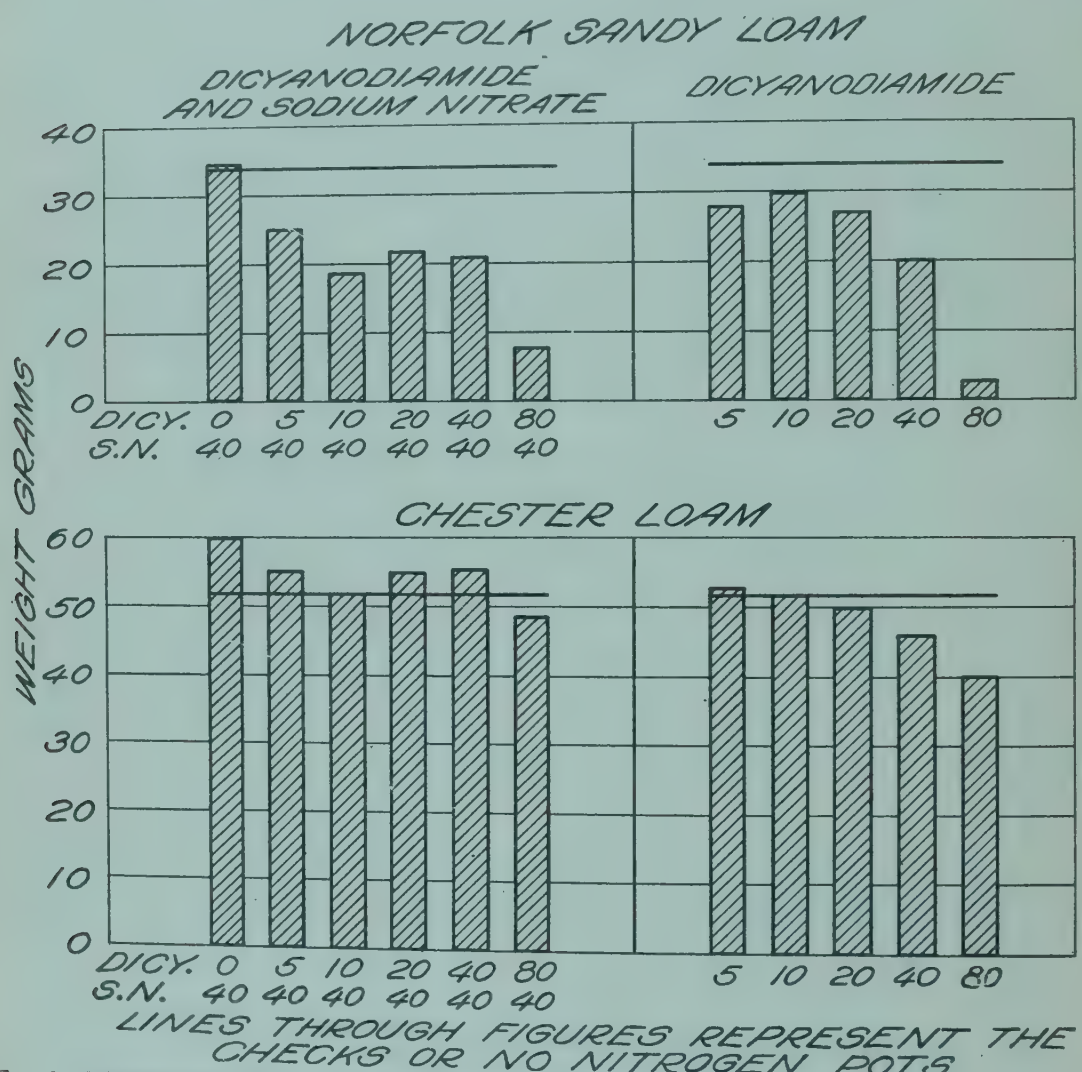

FIG. 2.—Diagram showing the yield of cowpeas from pots receiving various amounts of dicyanodiamide and sodium nitrate

The results with the Chester loam agree very closely with those in Table V in showing a fair increase in growth with sodium nitrate. Furthermore, the dicyanodiamide injury was slightly less in the presence of nitrate nitrogen, 10 pounds of the ammonia equivalent being sufficient to more than counteract 40 pounds of ammonia as dicyanodiamide. The leaf yellowing and other visible signs of the dicyanodiamide poisoning were not lessened in the presence of nitrate nitrogen.

marked difference between the toxicity of dicyanodiamide for wheat and for cowpeas was especially noticeable. The injury to wheat was only slight, even at the high concentration of 40 pounds of the ammonia equivalent per acre. For cowpeas, 5 pounds of ammonia as dicyanodiamide was decidedly toxic.

Where used on wheat, dicyanodiamide at the rate of 40 pounds of ammonia per acre produced some tip burning and decreased the green weights of the

plants 17 per cent on the Norfolk sandy loam and 13 per cent on the Chester loam. In the presence of sodium nitrate dicyanodiamide produced the tip burning but did not markedly decrease the value of sodium nitrate. This indicates that dicyanodiamide is not a marked direct poison for wheat but is merely unavailable as a plant food and probably prevents the proper utilization of the soil nitrogen.

Experiments to determine the residual effects of dicyanodiamide showed that the material remained in the pots and produced a marked decrease in the second crop (cowpeas) on the sandy soil whether previously used with or without sodium nitrate. On the loam soil the residual effects were almost negligible.

Additional experiments where cowpeas were grown in the presence of fresh

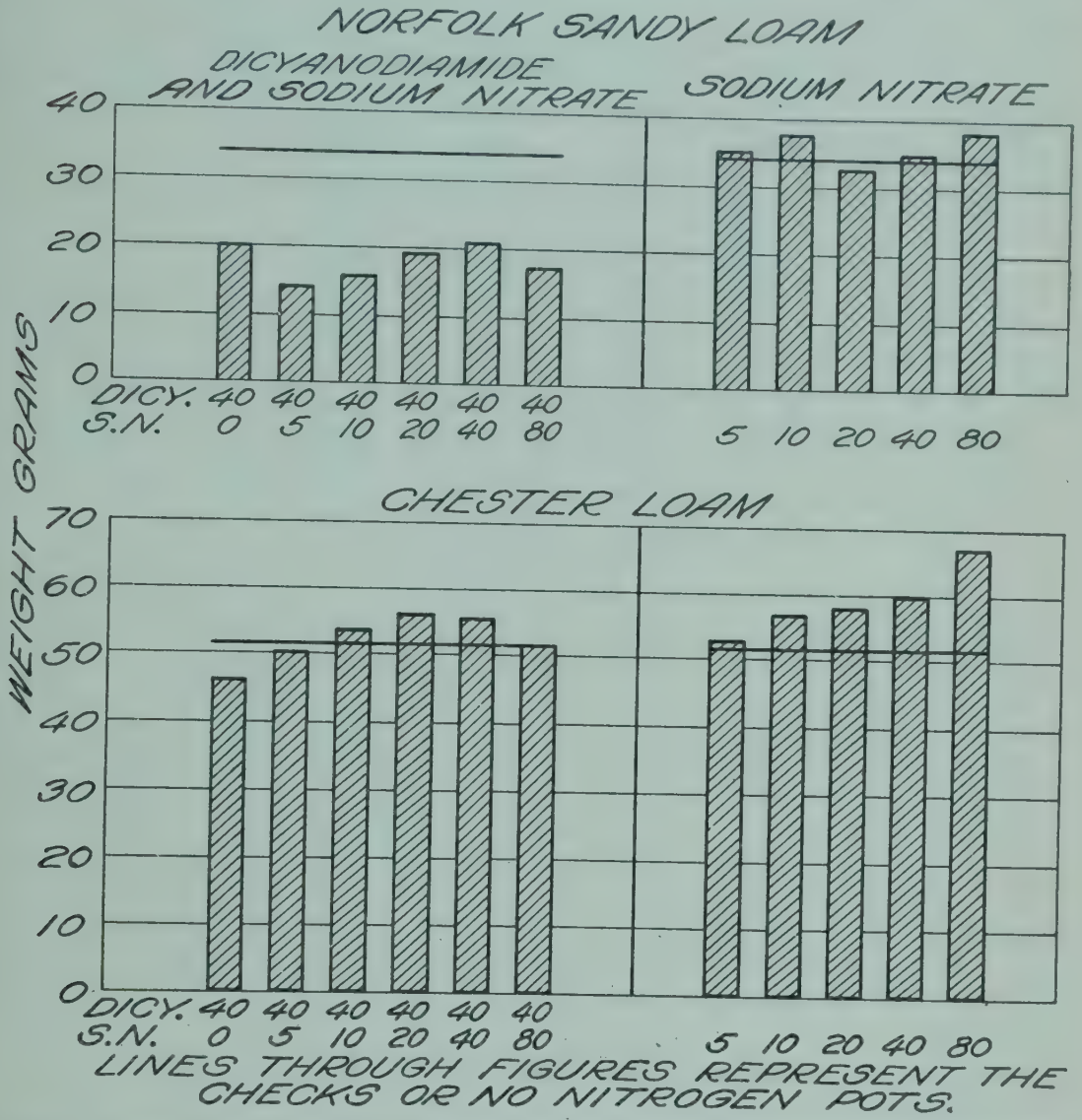

FIG. 3.—Diagram showing the yield of cowpeas from pots receiving various amounts of dicyanodiamide and sodium nitrate

In the presence of 40 pounds of ammonia as dicyanodiamide, sodium nitrate equivalent to 5 pounds of ammonia on the lighter soil and 10 pounds on the heavier was sufficient to counteract the decrease in the green weights of the wheat plants produced by the dicyanodiamide. Where sodium nitrate and dicyanodiamide were used in varying proportions, the green weights depended primarily upon the nitrate supply, the dicyanodiamide exerting only a slight injury and its nitrogen being unavailable.

applications of dicyanodiamide showed that this plant is injured even by small applications of the material (5 pounds of ammonia per acre). Sodium nitrate usually did not counteract the injury and even increased it in several instances. Practically all plants grown in the presence or absence of nitrate nitrogen showed a yellowing of the lower leaves and a slight mosaic appearance of the others. With the larger applications on the sandy soil germination was affected and the plants were nearly dead when the experiment was terminated.

TABLE V.—*Green weights of cowpeas from pots receiving various quantities of dicyanodiamide with and without sodium nitrate*

NORFOLK SANDY LOAM

Source of nitrogen	Fertilizer ratio NH_3–P_2O_5–K_2O	Green weight	Average	Variation from check	Nodule formation
		Grams.			
No nitrogen	0–8–4	32.6	34.1	----------	Abundant.
		35.5			Do.
Sodium nitrate 40	4–8–4	33.9	34.7	0.6	Do.
		35.5			Do.
Sodium nitrate 40, dicyanodiamide 5	4.5–8–4	24.9	25.0	−9.1	Many.
		25.1			Abundant.
Sodium nitrate 40, dicyanodiamide 10	5–8–4	17.1	18.4	−15.7	Many.
		19.7			Abundant.
Sodium nitrate 40, dicyanodiamide 20	6–8–4	17.2	21.6	−12.5	Many.
		25.9			Do.
Sodium nitrate 40, dicyanodiamide 40	8–8–4	20.9	20.9	−13.2	Do.
		25.8			Do.
		20.3			Do.
		16.6			Do.
Sodium nitrate 40, dicyanodiamide 80	12–8–4	4.3	7.1	−27.0	None.
		9.9			Many.
Dicyanodiamide 5	.5–8–4	27.4	27.7	−6.4	Do.
		27.9			Do.
Dicyanodiamide 10	1–8–4	33.3	29.8	−4.3	Abundant.
		26.3			Many.
Dicyanodiamide 20	2–8–4	23.9	2-.9	−7.2	Abundant.
		29.8			Do.
Dicyanodiamide 40	4–8–4	24.1	19.9	−14.2	Do.
		15.7			Many.
Dicyanodiamide 80	8–8–4	1.4	2.6	−31.5	None.
		3.8			Few.

CHESTER LOAM

No nitrogen	0–8–4	50.6	51.5	----------	Few.
		52.4			Do.
Sodium nitrate 40	4–8–4	58.6	60.1	8.6	Do.
		61.6			None.
Sodium nitrate 40, dicyanodiamide 5	4.5–8–4	56.4	55.0	3.5	Do.
		53.6			Few.
Sodium nitrate 40, dicyanodiamide 10	5–8–4	54.7	51.4	−.1	None.
		48.1			Few.
Sodium nitrate 40, dicyanodiamide 20	6–8–4	57.3	54.8	3.3	Do.
		52.3			None.
Sodium nitrate 40, dicyanodiamide 40	8–8–4	57.7	55.3	3.8	Few.
		53.8			None.
		53.7			Do.
		56.1			Few.
Sodium nitrate 40, dicyanodiamide 80	12–8–4	51.9	48.6	−2.9	Do.
		45.3			Do.
Dicyanodiamide 5	.5–8–4	51.6	52.9	1.4	Do.
		54.1			None.
Dicyanodiamide 10	1–8–4	54.1	51.9	.4	Few.
		49.7			Do.
Dicyanodiamide 20	2–8–4	47.1	50.0	−1.5	Do.
		52.8			Do.
Dicyanodiamide 40	4–8–4	42.9	46.0	−5.5	Do.
		49.0			None.
Dicyanodiamide 80	8–8–4	37.5	40.3	−11.2	Few.
		43.0			Do.

The difference between the extent of the injury of dicyanodiamide on wheat and cowpeas suggests an explanation for some of the widely varying results reported in the literature. For one crop the material may be very toxic, even in small amounts, while for other plants it is nearly inert or indirectly toxic because of its prevention of nitrification. This emphasizes the desirability of further work with other crops. However, dicyanodiamide was somewhat toxic even for the more resistant crop, wheat, and for this reason the material should not be present in appreciable quantities in fertilizers.

TABLE VI.—*Green weights of cowpeas from pots receiving various amounts of sodium nitrate with and without dicyanodiamide*

NORFOLK SANDY LOAN

Source of nitrogen	Fertilizer ratio NH_3-P_2O_5-K_2O	Green weight	Average	Variation from check	Nodule formation
		Grams			
No nitrogen	0-8-4	32.6	34.1	----------	Abundant.
		35.5			Do.
Dicyanodiamide 40	4-8-4	24.1	19.9	−14.2	Do.
		15.7			Many.
Dicyanodiamide 40, sodium nitrate 5	4.5-8-4	13.1	13.8	−20.3	Do.
		14.5			Do.
Dicyanodiamide 40, sodium nitrate 10	5-8-4	15.1	15.5	−18.6	Do.
		15.9			Do.
Dicyanodiamide 40, sodium nitrate 20	6-8-4	16.8	19.0	−15.1	Abundant.
		21.1			Many.
Dicyanodiamide 40, sodium nitrate 40	8-8-4	20.3	20.9	−13.2	Do.
		16.6			Do.
		20.9			Do.
		25.8			Do.
Dicyanodiamide 40, sodium nitrate 80	12-8-4	15.5	17.4	−16.7	Few.
		19.2			Many.
Sodium nitrate 5	.5-8-4	38.5	35.0	.9	Do.
		31.5			Do.
Sodium nitrate 10	1-8-4	36.5	37.7	3.6	Do.
		38.9			Do.
Sodium nitrate 20	2-8-4	31.9	32.8	−1.3	Do.
		33.7			Do.
Sodium nitrate 40	4-8-4	33.9	34.7	.6	Abundant.
		35.5			Do.
Sodium nitrate 80	8-8-4	37.6	38.3	4.2	Many.
		38.9			Do.

CHESTER LOAM

No nitrogen	0-8-4	50.6	51.5	----------	Few.
		52.4			Do.
Dicyanodiamide 40	4-8-4	42.9	46.0	−5.5	Do.
		49.0			None.
Dicyanodiamide 40, sodium nitrate 5	4.5-8-4	51.8	50.3	−1.2	Few.
		48.8			Do.
Dicyanodiamide 40, sodium nitrate 10	5-8-4	55.1	53.1	1.6	None.
		51.0			Few.
Dicyanodiamide 40, sodium nitrate 20	6-8-4	54.5	55.9	4.4	Do.
		57.3			None.
Dicyanodiamide 40, sodium nitrate 40	8-8-4	53.7	55.3	3.8	Do.
		56.1			Few.
		57.7			Do.
		53.8			None.
Dicyanodiamide 40, sodium nitrate 80	12-8-4	56.0	51.5	.0	Do.
		46.9			Do.
Sodium nitrate 5	.5-8-4	52.0	52.2	.7	Few.
		52.3			Do.
Sodium nitrate 10	1-8-4	57.7	56.3	4.8	Do.
		54.9			Do.
Sodium nitrate 20	2-8-4	60.8	57.3	5.8	None.
		53.8			Few.
Sodium nitrate 40	4-8-4	58.6	60.1	8.6	Do.
		61.6			None.
Sodium nitrate 80	8-8-4	66.9	66.2	14.7	Few.
		65.4			Do.

STUDIES ON THE SINGLE-INJECTION METHOD OF VACCINATION AS A PROPHYLACTIC AGAINST RABIES IN DOGS [1]

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INTRODUCTION

Immunization against rabies has been practiced for many years in both human and veterinary medicine. Its use has been confined almost exclusively to cases of exposure to rabies infection, and by means of the Pasteur and Högyes treatments or their modifications the mortality from the disease has been greatly reduced. These methods, while efficient, are not applicable as a practicable means of controlling rabies in dogs—the principal source of propagation of the disease—inasmuch as a number of injections are required with the consequent entailment of considerable expense.

Owing to the increase of rabies in Japan, Umeno and Doi [2] sought by means of vaccination a method for the control of the disease in dogs. After experimental work they concluded that it was possible to immunize dogs against rabies by means of one subcutaneous injection of glycerinated fixed virus which had been attenuated by the addition of phenol. This method was then put into practical use and large numbers of pet dogs in certain districts were given one dose of vaccine.

Umeno and Doi state:

> By summing up the statistics obtained in Kanagawa and Tokyo Prefectures we obtain the following figures:
>
> There were 31,307 vaccinated dogs, of which number there was only one case of vaccination loss and one in which the vaccination was not to be regarded effective enough. There appeared quite a number of rabid dogs among the nonvaccinated dogs. Thus, we see the great importance of the vaccination of dogs in order to prevent the spread of rabies among dogs.

Eichhorn and Lyon [3] and Reichel and Schneider [4] report experimental work with this method in the United States.

The striking results of the practical application of the single-injection vaccine, as reported by Umeno and Doi, in Japan, together with the experimental results reported by Eichhorn and Lyon in the United States in 1922, led to the receipt by the Bureau of Animal Industry, United States Department of Agriculture, of numerous letters from veterinarians, public health officials, and dog owners requesting information as to the efficiency of this method of immunization and its probable use in the control and eventual eradication of rabies from communities.

As rabies appears to be on the increase, particularly in certain sections of the country, and as the control of the disease is an important problem, not only from an economic point of view but also from a public-health standpoint, any method looking to the control of the disease in dogs deserves thorough consideration and study. Experimental work on this subject was therefore undertaken.

PREPARATION OF THE VACCINE

According to the method of Umeno and Doi, the vaccine is prepared as follows:

The brain and spinal cord of a rabbit dead of fixed rabies virus infection are removed and thoroughly ground in a mortar. To the ground-up mass four times its volume of phenolized glycerin water is added. The glycerin water consists of 60 parts of glycerin and 40 parts of water which contains 1.25 per cent phenol, making the glycerinated water contain 0.5 per cent phenol. After being thoroughly mixed, the emulsion is allowed to stand at room temperature of 18° to 22° C. for two weeks, or in the ice box for 30 days, after which time it is ready for use.

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[1] Received for publication June 16, 1924; issued May, 1925.

[2] UMENO, S., and DOI, Y. A STUDY ON THE ANTI-RABIC INOCULATION OF DOGS AND THE RESULTS OF ITS PRACTICAL APPLICATION. Kitasato Arch. Exp. Med. 4: 89–108, 1921.

[3] EICHHORN, A., and LYON, B. M. PROPHYLACTIC VACCINATION OF DOGS AGAINST RABIES. Jour. Amer. Vet. Med. Assoc. (n. s. 14) 61: 38–42. 1922.

[4] REICHEL, J., and SCHNEIDER, J. E. RABIES VACCINE CANINE. SINGLE DOSE TREATMENT. Jour. Amer. Vet. Med. Assoc. (n. s. 16) 63: 83–84. 1923.

This technic is followed more or less closely by the majority of the commercial houses manufacturing this product. In several instances, however, the fixed virus is killed by phenol, the resultant vaccine being a killed rather than an attenuated fixed virus.

SOURCE AND DOSAGE OF VACCINES

The vaccines used in the following experiments consisted of several lots prepared in the laboratory of the Bureau of Animal Industry, and of lots obtained from commercial houses manufacturing this product. The vaccines prepared in this laboratory were made according to the method of Umeno and Doi, with the attenuation taking place at room temperature for two weeks. The dosage was 3 c. c. for dogs under 20 pounds, 5 c. c. for dogs between 20 and 40 pounds, and 10 c. c. for dogs over 40 pounds.

The commercial vaccines were received in original sealed containers and were kept in the ice box until used. The dates on which the vaccines were used were well within the expiration dates marked on the outside containers, and the dose given was according to the manufacturers' directions.

EXPERIMENTAL DATA

METHOD OF INJECTION

The loose skin back of the shoulder or elbow of the dog was shaved, the skin disinfected with 3 per cent phenol, dried, and painted with 3.5 per cent tincture of iodine. The proper amount of vaccine was then slowly injected subcutaneously in two or three places.

After varying intervals of time the vaccinated dogs, together with a number of control dogs, received an intraocular injection of rabies street virus.

EFFECT OF THE INJECTION OF VACCINE

The dogs were not inconvenienced by the injection of the vaccine. They remained lively and ate heartily at all times. In one case, however, the injection of the vaccine resulted in a death from rabies. Dog 440 was injected with 5 c. c. of Bureau of Animal Industry vaccine No. 1 on May 25, 1922. On September 13, 1922, the dog showed signs of paralysis and on September 14 was stretched out on its side, completely paralyzed. It continued in this condition through September 15, and was found dead on the morning of September 16. Microscopic examination of smears from the hippocampus revealed the presence of a few small Negri bodies.

Three rabbits inoculated subdurally with brain material from dog 440 were completely paralyzed on the seventh day after inoculation and died two to three days later. Microscopic examination of the brains of these three rabbits revealed the presence of a few small Negri bodies of the type usually seen in fixed virus.

Reinoculation of rabbits with the brain of one of these rabbits was followed by typical rabies paralysis in the inoculated animals on the seventh day.

Several more passages of this virus were made in rabbits, and in every instance complete paralysis occurred on the seventh day.

These inoculation results clearly indicate that dog 440 died of rabies fixed-virus infection. Three other dogs received the same amount of this vaccine and on the same day as dog 440, but remained perfectly healthy. This was the only case of death as a result of the injection of the vaccine.

METHOD OF EXPOSURE TO STREET VIRUS

One of the difficulties encountered in experimental work on rabies immunization is the question of the mode of exposure and the amount of street virus to be injected. It is extremely difficult or impossible to standardize street virus, owing to the variable action of the same amount of virus on different individuals. Probably with no other disease does individual resistance play so important a part as in rabies. What is highly virulent for one dog may not be sufficient to cause the development of the disease in another.

For an experiment to be successful the exposure to street virus should be sufficiently virulent to cause the development of the disease, if not in all, at least in the great majority of the control dogs. On the other hand, the virus should not be so virulent as to be simply overwhelming.

The intraocular injection is a severe test, probably much more so than an animal would encounter naturally. However, as an experimental means of exposure it is probably better than any other method. Umeno and Doi, Eichhorn and Lyon, and Reichel and Schneider report the use of this method in experimental work, and it is the method used in the following experiments.

EXPERIMENT NO. 1

On May 25, 1922, 7 dogs each received one injection of vaccine. On July 18, 11

additional dogs each received one injection, making a total of 18 vaccinated dogs. Nine of these were given a vaccine made in this laboratory (designated B. A. I.) and 9 were vaccinated with vaccines prepared by three commercial firms (designated respectively A, B, and C.). On September 12, 1922, the 18 vaccinated dogs together with 8 control dogs received an injection of 0.1 c. c. of rabies street virus in the anterior chamber of the left eye. The virus consisted of a 1:10 dilution in salt solution of the hippocampus and medulla of a dog dead of rabies.

The rabies street virus used in experiment No. 1 was obtained from a dog which developed the disease spontaneously at the Bureau of Animal Industry Experiment Station, Bethesda, Md. This dog, No. 474, was purchased from the District of Columbia pound on August 22, 1922, and was being held for use as a control or check dog in a rabies experiment. On September 10 the animal developed clinical symptoms of furious rabies and was found dead September 11. The diagnosis of rabies was confirmed by microscopic examination and subsequently by rabbit inoculations. On September 12 a 1:10 dilution of the hippocampus and medulla of this dog was made in salt solution and used as the exposure virus in experiment No. 1. This virus was designated Bureau of Animal Industry No. 474.

The data of experiment No. 1 are given in Table I.

TABLE I.—*Data of experiment No. 1*

Dog No.	Vaccine [a]	Amount injected	Date vaccine was injected	Date exposed to virus [b]	Result [c]	Date of rabbit inoculation	Result
		C. c.					
1	A	5	May 25, 1922	Sept. 12, 1922	Dead Nov. 2, 1922; rabies.	Nov. 2, 1922	Dead, Nov. 16, 1922; rabies.
2	A	5	do	do	Dead, Sept. 30, 1922; rabies.	Sept. 30, 1922	Dead, Oct. 14, 1922; rabies.
3	B	5	do	do	Dead, Sept. 29, 1922; rabies.	do	Dead Oct. 15, 1922; rabies.
4	B. A. I.	5	do	do	Dead, Oct. 2, 1922; rabies.	Oct. 3, 1922	Do.
5	B. A. I.	5	do	do	Alive, Mar. 7, 1923.		
6	B. A. I.	5	do	do	Dead, Sept. 29, 1922; rabies.	Sept. 30, 1922	Do.
7	B	5	do	do	Alive, Mar. 7, 1923.		
8	A	5	July 18, 1922	do	Dead, Sept. 24, 1922; rabies.	Sept. 27, 1922	Dead, Oct. 17, 1922; rabies.
9	B. A. I.	3	do	do	Dead, Sept. 26, 1922; rabies.	do	Dead, Oct. 9, 1922; rabies.
10	B. A. I.	5	do	do	Alive, Mar. 7, 1923.		
11	B. A. I.	5	do	do	Dead, Sept. 28, 1922; rabies.	Sept. 30, 1922	Dead, Oct. 15, 1922; rabies.
12	B. A. I.	3	do	do	Dead, Sept. 25, 1922; rabies.	Sept. 28, 1922	Dead, Oct. 9, 1922; rabies.
13	C	5	do	do	Dead, Sept. 24, 1922; rabies.	Sept. 27, 1922	Dead, Oct. 11, 1922; rabies.
14	B. A. I.	5	do	do	do	Sept. 26, 1922	Dead, Oct. 9, 1922; rabies.
15	B. A. I.	5	do	do	do	Dec. 7, 1922	Dead, Dec. 21, 1922; rabies.
16	B	5	do	do	do	Sept. 26, 1922	Dead, Oct. 6, 1922; rabies.
17	B	5	do	do	Dead, Sept. 25, 1922; rabies.	Sept. 28, 1922	Dead, Oct. 17, 1922; rabies.
18	B	3	do	do	Dead, Sept. 28, 1922; rabies.	do	Dead, Oct. 11, 1922; rabies.
19	Control dog.			do	Dead, Oct. 24, 1922; enteritis.	Oct. 24, 1922	Alive, Dec. 24, 1922.
20	do			do	Alive, Jan. 16, 1923.		
21	do			do	do		
22	do			do	do		
23	do			do	do		
24	do			do	do		
25	do			do	do		
26	do			do	Dead, Oct. 9, 1922; rabies.		

[a] Vaccines A, B, and C were from three different commercial houses manufacturing this product. Vaccine B. A. I. was prepared in the laboratory of the Bureau of Animal Industry.

[b] The virus consisted of a 1 : 10 dilution in salt solution of the hippocampus and medulla of dog 474, dead of rabies; 0.1 c. c. of this material was injected into the anterior chamber of the left eye.

[c] The diagnosis of rabies was made by microscopic examination, and in the case of vaccinated dogs by rabbit inoculations. The surviving control dogs were destroyed Jan. 16, 1923, and the surviving vaccinated dogs were destroyed Mar. 7, 1923.

Table I may be summarized as follows: Eighteen dogs received one injection of vaccine, 7 on May 25 and 11 on July 18. On September 12 the 18 vaccinated dogs together with 8 control dogs received an intraocular injection of street virus. Of the 18 vaccinated dogs, 15 died of rabies. Of the 8 control dogs 1 died of rabies and 1 of enteritis.

In this and the following experiments the diagnosis of rabies in vaccinated animals was always confirmed by microscopic examination and rabbit inoculations.

The irregular outcome of this experiment is rather difficult of interpretation. The first thought that came to mind was the possibility of the vaccine sensitizing the animals, so that they succumbed to infection more quickly than nonvaccinated dogs. Subsequent experiments have not proved this to be the case, however, and the following is probably the cause of the irregularity of the result: The 18 vaccinated dogs were housed in one barn, and these were the first to be exposed to the street virus. The 8 control dogs were housed in a second barn about one-fourth of a mile from the first. After the dogs in the first barn had been exposed the virus was carried in a small beaker to the second barn, where the control dogs were then injected. Exposure to sunlight or to some unknown factor probably caused an alteration of the virus between the first and second barns. The injections in both groups of animals were made by the writer, and the material was stirred well before each injection.

Although the result of experiment No. 1 was irregular, it showed conclusively that the single injection of a vaccine did not produce sufficient immunity to protect dogs against an intraocular injection of the street virus used.

EXPERIMENT NO. 2

It was decided to repeat the previous experiment. Three dogs were vaccinated on July 18 and three on October 11, 1922. On October 26 these six dogs, together with three control dogs, received an intraocular injection of rabies street virus, 0.1 c. c. of a 1:10 dilution of the hippocampus and medulla of dog 26, dead of rabies (B. A. I. virus 474). The results are given in Table II.

Summarizing Table II it appears that five of the six vaccinated dogs died of rabies. The death of the sixth could not be attributed to rabies. All three controls died of rabies.

The diagnosis of rabies in the vaccinated animals was confirmed by microscopic examination and rabbit inoculations. In the control dogs the diagnosis was made clinically and by the demonstration of Negri bodies microscopically.

The incubation period of the disease was about the same in the vaccinated dogs as in the controls, showing that

TABLE II.—*Data of experiment No. 2*

Dog No.	Vaccine [a]	Amount injected	Date vaccine was injected	Date exposed to virus [b]	Result [c]	Date of rabbit inoculation	Result
		C. c.					
27	B	5	July 18, 1922	Oct. 26, 1922	Dead, Dec. 23, 1922; rabies.	Dec. 27, 1922	Dead, Jan. 25, 1923; rabies.
28	C	3	do	do	Dead, Nov. 21, 1922; rabies.	Nov. 23, 1922	Dead, Dec. 7, 1923; rabies.
29	A	5	do	do	Dead, Nov. 15, 1922; rabies.	Nov. 16, 1922	Dead, Dec. 26, 1923; rabies.
30	B. A. I.	5	Oct. 11, 1922	do	Dead, Nov. 24, 1922; rabies.	Nov. 25, 1922	Dead, Dec. 12, 1923; rabies.
31	do	3	do	do	Dead, Dec. 8, 1922; rabies.	Dec. 12, 1922	Dead, Dec. 26, 1923; rabies.
32	do	5	do	do	Died suddenly, Dec. 14, 1922; not rabies.	Dec. 15, 1922	Alive, Feb. 15, 1923.
33	Control dog.			do	Dead, Nov. 24, 1922; rabies.		
34	do			do	Dead, Nov. 10, 1922; rabies.		
35	do			do	Dead, Dec. 14, 1922; rabies.		

[a] Vaccines A, B, and C were from three different commercial houses manufacturing this product. Vaccine B. A. I. was prepared in the laboratory of the Bureau of Animal Industry.

[b] The virus consisted of a 1:10 dilution in salt solution of the medulla and hippocampus of dog 26 (B. A. I 474); 0.1 c. c. of this material was injected into the anterior chamber of the left eye.

[c] Diagnosis of rabies was made by microscopic examination and, in the case of vaccinated dogs, by rabbit inoculations.

the vaccination had no appreciable effect on the incubation period of the disease. If the period of incubation in the control animals can be used as an index of the virulence of the virus, it appears that the animals were not overwhelmed with virus.

This experiment confirmed the conclusions drawn from experiment No. 1, namely, that a single injection of vaccine failed to produce sufficient immunity to protect dogs against an intraocular injection of street virus B. A. I. 474.

The results of the two foregoing experiments were in contradiction to the results obtained by Eichhorn and Lyon, who protected 100 per cent of their animals against an intraocular injection of street virus.

In comparing experimental data of Eichhorn and Lyon with the preceding experiments, it was observed that the only practical differences between the experiments were the nature of the exposure virus used and the personal factor of technic. Leaving the personal factor aside, the only difference remaining was the use of a different exposure virus. It was considered advisable at this time to inquire into the source of the fixed virus used by the various commercial houses manufacturing the single-injection canine rabies vaccine.

As a result of this inquiry it was learned with considerable surprise that the virus used by all the commercial houses apparently had a common origin, the Pasteur Institute of Paris, France. The canine vaccines manufactured in this country, therefore, are prepared with a European strain of fixed virus. As a number of the commercial houses manufacturing canine rabies vaccine also prepare rabies vaccine for human use, it is probable that the fixed virus used for this product also had the same source.

There was a possibility, it was believed, that there might be different strains of rabies street virus against which, or some of which, protection could not be obtained by immunization with the strain of fixed virus used as a vaccine. To determine this point was the object of experiment No. 3.

EXPERIMENT NO. 3

Eighteen dogs were given one injection of vaccine. They were then divided into three groups of six each, and to each group were added four normal dogs to be used as controls.

Each group of 10 dogs (6 vaccinated and 4 controls) was exposed to an intraocular injection of a street virus of different origin.

Virus No. 1 had the following history: Pennsylvania dog to man, to rabbit, to rabbit, to dog; 0.1 c. c. of a 1:2 dilution of the brain of the dog was used for exposure.

Virus No. 2 was the B. A. I. 474 strain, a 1 : 10 dilution of the medulla of dog No. 35, dead December 14, and in glycerin since that time.

Virus No. 3 consisted of small pieces of rabid brain material of a New York dog, cat, and calf. This material, however, had been in glycerin for a considerable period of time.

The results of this experiment are given in Table III.

Table III may be summarized as follows: In lot No. 1, exposed to virus No. 1, one vaccinated dog died of rabies and five survived. Of the four control dogs, all died of rabies. Sixteen and two-thirds per cent of the vaccinated dogs died, as against 100 per cent of the control dogs, indicating that considerable protection was afforded by vaccination against this virus.

In lot No. 2, exposed to B. A. I. 474 virus, four vaccinated dogs died of rabies and two survived. Of the four control dogs, three died of rabies and one survived. Sixty-six and two-thirds per cent of the vaccinated dogs died, as against 75 per cent of the control dogs. This confirms the previous experiment in which little or no protection was afforded by vaccination against this virus.

Attention is called to dog 47 in this lot, which showed symptoms of furious rabies March 5 and was found dead on the morning of March 6. Extracellular Negri bodies of average size were found. Rabbits inoculated with brain material of this dog were completely paralyzed on the seventh day. Four passages of this virus through rabbits resulted in each instance in the development of complete paralysis on the seventh day. Negri bodies in these rabbits were scarce, but of very fair size. These results indicate that the virus in the brain of dog 47 had the properties of a fixed virus. Whether the exposure virus became fixed for the rabbit after passage through this dog, or whether the animal succumbed to fixed-virus infection from the vaccine which became exalted as a result of the injection of the street virus, are questions difficult to answer.

In lot No. 3, exposed to virus No. 3, one vaccinated dog died of rabies after an incubation period of almost four

TABLE III.—*Data of experiment No. 3*

LOT NO. 1

Dog No.	Vaccine [a]	Amount injected	Date vaccine was injected	Date exposed to virus [b]	Result [c]	Date of rabbit inoculation	Result
		C. c.					
36	B	5	Nov. 18, 1922	Feb. 1, 1923	Alive, Nov. 1, 1923.		
37	C	5	Dec. 13, 1922	do	do		
38	B. A. I.	5	Nov. 28, 1922	do	do		
39	A	5	Nov. 21, 1922	do	do		
40	do	5	Nov. 28, 1922	do	Dead, Feb. 16, 1923; rabies.	Feb. 17, 1923	Dead, Feb. 28, 1923; rabies.
41	D	5	Nov. 21, 1922	do	Alive, Nov. 1, 1923.		
42	Control dog			do	Dead, Apr. 5, 1923; rabies.	Apr. 7, 1923	Dead, Apr. 25, 1923; rabies..
43	do			do	Dead, Mar. 17, 1923; rabies.		
44	do			do	Dead, June 7, 1924.	June 9, 1924	Dead, June 25, 1924; rabies.
45	do			do	Dead, Feb. 22, 1923; rabies.		

LOT NO. 2

46	B	5	Nov. 21, 1922	Feb. 20, 1923	Dead, Mar. 10, 1923; rabies.	Mar. 24 1923	Dead, Apr. 4, 1923; rabies.
47	C	5	Dec. 13, 1922	do	Dead, Mar. 6, 1923; rabies.	Mar. 8, 1923	Dead, Mar. 16, 1923; rabies.
48	B. A. I.	5	Nov. 28, 1922	do	Killed, May 10, 1923; by dog 39.		
49	do	5	do	do	Dead, Apr. 2, 1923; rabies.	Apr. 3, 1923	Dead, Apr. 16 1923; rabies.
50	A	5	Nov. 21, 1922	do	Alive, Nov. 1, 1923.		
51	D	5	do	do	Dead, Apr. 9, 1923; rabies.	Apr. 16, 1923	Dead, Apr. 27, 1923; rabies.
52	Control dog.			do	Alive, Nov. 1, 1923.		
53	do			do	Dead, Mar. 6, 1923; rabies.		
54	do			do	Dead, Mar. 5, 1923; rabies.		
55	do			do	Dead, Mar. 6, 1923; rabies.		

LOT NO. 3

56	B	5	Nov. 28, 1922	Feb. 21, 1923	Alive, Nov. 1, 1923.		
57	do	5	Dec. 13, 1922	do	do		
58	B. A. I.	5	Nov. 28, 1922	do	do		
59	A	5	Jan. 13, 1922	do	do		
60	D	5	Nov 28, 1922	do	do		
61	do	5	Dec. 13, 1922	do	Dead, June 16, 1923; rabies.	June 18, 1923	Dead, June 29, 1923; rabies.
62	Control dog.			do	Killed, June 19, 1923; by dog 56.		
63	do			do	Alive, Nov. 1, 1923.		
64	do			do	do		
65	do			do	do		

[a] Vaccines A, B, C, and D were from four different commercial houses manufacturing this product. B. A. I. vaccine was prepared in the laboratory of the Bureau of Animal Industry.

[b] Lot No. 1 was exposed to virus No. 1, lot No. 2 to virus No. 2, and lot No. 3 was exposed to virus No. 3. In lots Nos. 1 and 3 the virus consisted of a 1:10 dilution of the hippocampus and medulla of a dog dead of rabies of a different source. Lot No. 2 consisted of a 1:2 dilution of the medulla and hippocampus. One-tenth of a cubic centimeter was injected into the anterior chamber of the eye.

[c] Diagnosis of rabies was made by microscopic examination, and in the case of vaccinated dogs and control dogs 42 and 44 also by rabbit inoculations.

months. None of the remaining vaccinated dogs nor any of the control dogs developed the disease. The small pieces of brain material composing this virus and the length of time in glycerin evidently led to its deterioration. It is significant to note, however, that the only animal to succumb in this lot was a vaccinated one. This experiment appears to indicate that a certain amount of immunity can be produced by a single injection of vaccine against one strain of virus, but practically none against another strain.

EXPERIMENT NO. 4

The fourth experiment was a repetition of the previous experiments, except that a still different street virus was used for exposure.

The source of the street virus was Washington, D. C.; dog to rabbit, to dog 630. A 1:10 dilution of the hippocampus and medulla of dog 630 was used for exposure virus.

The results are given in Table IV.

Table IV may be summarized as follows: Ten dogs were given a single injection of vaccine, and later, together with 6 control dogs, were given an intraocular injection of street virus from dog 630. Of the 10 vaccinated dogs 3, or 30 per cent, died of rabies, while of the 6 control dogs all succumbed to the disease.

In this experiment distinct protection was afforded by a single injection of vaccine. All the control animals succumbed to the disease with a short incubation period, which probably indicates a high degree of virulence for the exposure virus and a severe test on the efficiency of the method of vaccination.

EXPERIMENT NO. 5

The fifth experiment had for its object the exposure of vaccinated and nonvaccinated dogs to the bites of a rabid animal.

Dogs 56, 57, 58, and 59 were vaccinated animals, and dogs 63, 64, and 65 were controls used in experiment No. 3, lot No. 3, which had failed to develop rabies after intraocular exposure. Dogs 82 and 83 were normal dogs used as controls.

The dog to which the abovementioned animals were exposed was the one which after death furnished the virus for exposure in experiment No. 4. The source of the virus was

TABLE IV.—*Data of experiment No. 4*

Dog No.	Vaccine [a]	Amount injected	Date vaccine was injected	Date exposed to virus [b]	Result [c]	Date of rabbit inoculation	Result
		C. c.					
66	A	5	June 14, 1923	Nov. 20, 1923	Alive, April 20, 1924.		
67	B	5	do	do	do		
68	A	5	do	do	do		
69	C	5	Aug. 4, 1923	do	do		
70	C	5	do	do	do		
71	D	5	do	do	do		
72	B	5	Sept. 5, 1923	do	Dead, Jan. 17, 1924; rabies.	Feb. 4, 1924	Dead, Feb. 19, 1924; rabies.
73	B	5	do	do	Dead, Dec. 26, 1923; rabies.	Dec. 27, 1923	Dead, Jan. 11, 1924; rabies.
74	D	5	do	do	Dead, Dec. 10, 1923; rabies.	Dec. 11, 1923	Dead, Dec. 27, 1923; rabies.
75	D	5	do	do	Alive, Apr. 20, 1924.		
76	Control dog.			do	Dead, Dec. 11, 1923; rabies.		
77	do			do	Dead, Dec. 8, 1923; rabies.		
78	do			do	Dead, Dec. 12, 1923; rabies.		
79	do			do	Dead, Dec. 11, 1923; rabies.		
80	do			do	Dead, Dec. 10, 1923; rabies.		
81	do			do	Dead, Dec. 7, rabies.		

[a] Vaccines A, B, C, and D were from four different commercial houses manufacturing this product.

[b] The virus consisted of a 1:10 dilution of the medulla and hippocampus of dog 630 dead of rabies; 0.1 c. c. of this material was injected into the anterior chamber of the left eye.

[c] Diagnosis of rabies was made by microscopic examination, and in vaccinated dogs was also confirmed by rabbit inoculations.

Washington, D. C.; dog to rabbit to dog 630. Dog 630, the animal used, was inoculated intraocularly on October 24, 1923. On November 12, 1923, the animal presented clinical sumptoms of rabies.

Each dog in the experiment was placed in the same cage with dog 630. In each instance the animals were attacked and bitten by the rabid dog. Each animal was left in the cage until it was definitely seen that wounds were inflicted. The effort was made to give all the dogs the same degree of exposure. The results of the test are given in Table V.

Against a still different strain of street virus (B. A. I. 474), however, practically no protection was afforded to vaccinated animals exposed by intraocular injection. Of 30 vaccinated dogs exposed to this virus, 6 survived.

A study of the vaccines indicates that the source, age, etc., had no bearing on the outcome of the various experiments. All the vaccines used in these experiments were prepared from the same strain of fixed virus, which had its origin in the Pasteur Institute of Paris, France. The results of the experiments herein reported indicate that this strain of fixed virus, when used as a vaccine, protects against certain strains of street virus, but that one strain of street virus was encountered against which no protection could be obtained.

TABLE V.—*Data of experiment No. 5*

Dog No.	Vaccine [a]	Amount injected	Date vaccine was injected	Date exposed to virus [b]	Result [c]
		C. c.			
56	B	5	Nov. 28, 1922	Nov. 12, 1923	Alive Apr. 12, 1924.
57	B	5	Dec. 13, 1922	do	Do.
58	B. A. I.	5	Nov. 28, 1922	do	Do.
59	A	5	Jan. 13, 1922	do	Do.
63				do	Dead Dec. 3, 1923; rabies.
64				do	Alive Apr. 12, 1924.
65				do	Do.
82				do	Do.
83				do	Dead Dec. 3, 1923; rabies.

[a] Vaccines A and B were from two different commercial houses manufacturing this product. Vaccine B. A. I. was prepared in the laboratory of the Bureau of Animal Industry.
[b] These animals were exposed to the bites of a rabid dog, No. 630.
[c] Diagnosis of rabies made clinically and by microscopic examination.

Experiment No. 5 may be summarized as follows: Four vaccinated dogs and five control dogs were exposed to the bites of a rabid animal; two of the five controls succumbed to rabies. None of the vaccinated animals developed the disease.

The number of animals exposed to rabies and not treated in which infection developed has been estimated by various investigators to be between 16 and 60 per cent. In this experiment 40 per cent of the nonvaccinated animals bitten developed the disease. In view of the fact that none of the vaccinated animals developed the disease, it can be assumed that the vaccine afforded protection in this experiment.

DISCUSSION

From the foregoing experiments it appears that the efficacy of the single injection canine rabies vaccine as a prophylactic depends on the street virus to which the vaccinated animal is exposed. Against intraocular injections of two different strains of street virus, distinct protection was afforded by the vaccine, 12 out of the 16 vaccinated dogs being protected.

The question as to whether these results are due to a difference in virulence of the virus used and not to a distinct difference of strain can be answered, it is believed, by a comparison of experiments Nos. 2 and 4.

In experiment No. 2, using the strain of virus B. A. I. 474, the three control dogs died of rabies in 15, 29, and 49 days. Assuming the incubation period of the disease to be an index to the virulence of the virus, it would appear that this virus was not extremely virulent.

In experiment No. 4, 1 of the 6 control dogs showed symptoms of rabies on the sixteenth day, 4 on the seventeenth day, and 1 on the twentieth day. This incubation period would indicate a higher degree of virulence for this virus than for B. A. I. 474, yet 7 out of 10 dogs were protected against this virus, while 5 out of 6 dogs exposed to B. A. I. 474 virus died of rabies. These facts point to the possibility of the existence in this country of more than one strain of street virus.

In this event the extent to which the single-injection vaccine will assist in the control and eradication of rabies in dogs will depend in a large measure on the extent and distribution of the strain of virus designated B. A. I. 474 or similar strains, until such time as polyvalent vaccines can be prepared from more than the one strain of fixed virus as is now the case.

There is a possibility that the injection of a vaccine prepared according to the method of Umeno and Doi may result in death from fixed-virus infection, as seen in the case of dog 440.

The subcutaneous injection of fixed virus, even without attenuation, is generally considered to be harmless. This is undoubtedly true in general, but occasionally an individual may be encountered that is extremely susceptible and infection may result, even with an attenuated fixed virus. The percentage of such individuals, however, is believed to be small.

Umeno and Doi state that of the 31,307 vaccinated dogs there was one case of vaccination loss. It is presumed from this statement that the loss was due to fixed-virus infection following vaccination.

SUMMARY

The foregoing work may be summarized as follows:

The virus to which vaccinated dogs were exposed was the factor which determined the efficacy of the single-injection method of vaccination.

Against one virus no protection was afforded vaccinated animals.

Against two other viruses distinct protection was afforded.

The vaccines prepared in this country by commercial houses are all made from a strain of fixed virus having its origin in the Pasteur Institute of Paris, France.

There appears to be more than one strain of rabies street virus in this country.

THE STRAWBERRY ROOT LOUSE IN TENNESSEE[1]

By S. MARCOVITCH

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INTRODUCTION

In Tennessee, strawberries are classed as nursery stock and before they can be shipped an inspection is required for injurious insects, particularly for the strawberry root louse (*Aphis forbesi* Weed). Since no extended study of this insect in Tennessee had ever been made and since little is known concerning it in other States, except in Delaware, it seemed desirable to make a study of its habits and to determine if possible its economic importance. When one considers the vast quantities of strawberries grown in Tennessee, which is the largest strawberry-producing State in the Union, and the time consumed each year inspecting them, the need of a more definite knowledge of the economic importance of this insect becomes apparent.

GEOGRAPHIC RANGE

The strawberry root louse is a native insect confined to eastern North America, and has been reported injurious in Illinois, Ohio, Maryland, Tennessee, and Delaware, although it has recently been learned through correspondence that it is no longer so considered in the last-named State. In Tennessee it is present wherever strawberries are grown.

HOST PLANTS

The cultivated strawberry, *Fragaria* sp. appears to be the only host of *Aphis forbesi* Weed. Repeated efforts have been made to find it on other plants, but without success. Often the roots of various weeds are infested with lice very similar in appearance to the strawberry root louse, but close examination reveals them to be other species, usually the corn root louse, *Aphis maidiradicis*.

ECONOMIC IMPORTANCE

Plant lice in general are of great economic importance, yet there are many species that do little or no harm.

To obtain exact data in regard to injury caused by *Aphis forbesi* Weed, experiments were conducted in the field during 1921 and 1922. The vigor of a strawberry plant is indicated by the number of plants and runners which it produces. Plants artificially infested with lice were set out in March and a record was kept of the number of plants and runners produced. The plants used in the control plat were freed from any lice that may have been present by dipping them in a tobacco solution. The control plat was also frequently sprayed with tobacco to guard against infestation. The results obtained for the year 1922 are as follows: Of 12 infested mother plants the maximum number of plants produced by any one was 150, the minimum 12, and the average 47.6. Of 22 control mother plants the individual maximum was 120, the minimum 4, and the average 49.7 plants. The mother plants were set March 28 and the count of plants produced was made September 16, 1922. The difference in the percentage of plants produced by the infested mother plants and the controls was small, and could easily come within the limits of experimental error.

Practically the same results were obtained in 1921. Ladybird beetles often destroyed the lice on the crown of the plant, where they are frequently found. In such cases the plants were artificially reinfested.

To ascertain the general importance of the strawberry root louse a circular letter was sent to all the important strawberry-producing States. Replies from Iowa, Illinois, Ohio, New Jersey, Delaware, North Carolina, Georgia, Florida, Louisiana, Mississippi, and Arkansas indicated that the strawberry root louse was not considered injurious to the strawberry, or that little consideration was given to it. The only State reporting serious injury from this pest was Maryland, where it is considered "highly undesirable" in the light sandy soils, although no data were given. The strawberry soils of Ten-

[1] Received for publication June 11, 1924, issued, May, 1925.

nessee are mostly loams or silt loams, which may be one reason why the insect is less injurious in this State.

LIFE HISTORY

Unlike some other species of plant lice, the strawberry root louse spends its entire life cycle upon the strawberry, migrating only to other strawberry fields.

THE EGG

The insect passes the winter in the egg stage upon the pedicels of the leaves (pl. 1,₀), although eggs are sometimes laid on the underside of the leaves. In 1921, at Knoxville, Tenn., hatching began on February 15, and in 1922 on February 22; hatching continues for about a month.

FIRST INSTAR (PL. 2, G)

Color characters: Upon hatching the new-born nymphs are pale green in color, with legs, antennae, and cornicles whitish. After a few hours the general coloration becomes greenish; head lighter, with two chitinized areas on either side of the median line; eyes reddish; antennae pale yellow, darker apically; tip of beak dusky; legs pale yellow; tarsi dusky; cornicles pale, darker at the tip.

Morphological characters: Body rectangular, flattened; antennae and legs robust as compared with the rest of the body; antennae four-jointed, with a sensorium present at the distal end of the third joint and at the proximal end of the flagellum of the fourth joint; cornicles short, about as broad as long.

Measurements: Length, 0.52 to 0.60 mm.; width, 0.28 to 0.30 mm.; antennae, 0.26 mm. (pl. 3, J); segment No. 1, 0.03 mm.; segment No. 2, 0.03 mm.; segment No. 3, 0.08 mm.; segment No. 4, 0.12 mm.; cornicles (pl. 3, K), length, 0.03 mm.; width, 0.03 mm.

SECOND INSTAR

This stage does not differ in general coloration from that of the first stage. The body becomes ovate in form and the antennae five-segmented. The cornicles also become more elongate, being about twice as long as wide.

Measurements: Length, 0.82 to 0.92 mm.; width, 0.40 to 0.44 mm.; antennae, 0.31 mm; segment No. 1, 0.03 mm.; segment No. 2, 0.03 mm.; segment No. 3, 0.06 mm.; segment No. 4, 0.04 mm.; segment No. 5, 0.15 mm.; cornicles, length, 0.05 mm.

STEM-MOTHER

After passing through five instars the stem-mother becomes mature in two to three weeks, depending upon the temperature. In 1921 stem-mothers were full grown as early as March 8. Upon maturity, often only a few hours after the last molt, they give birth to living young. In a study of the reproductive capacity of the stem-mothers, it was found that they were capable of giving birth to an average of two young per day for a period of 20 to 28 days. The highest number of young produced in one day was six.

The intitial feeding of the young stem-mothers takes place where they hatch, but after a short time they find their way to the tenderest leaves just coming out of the crown. Here they insert their beaks and suck the sap.

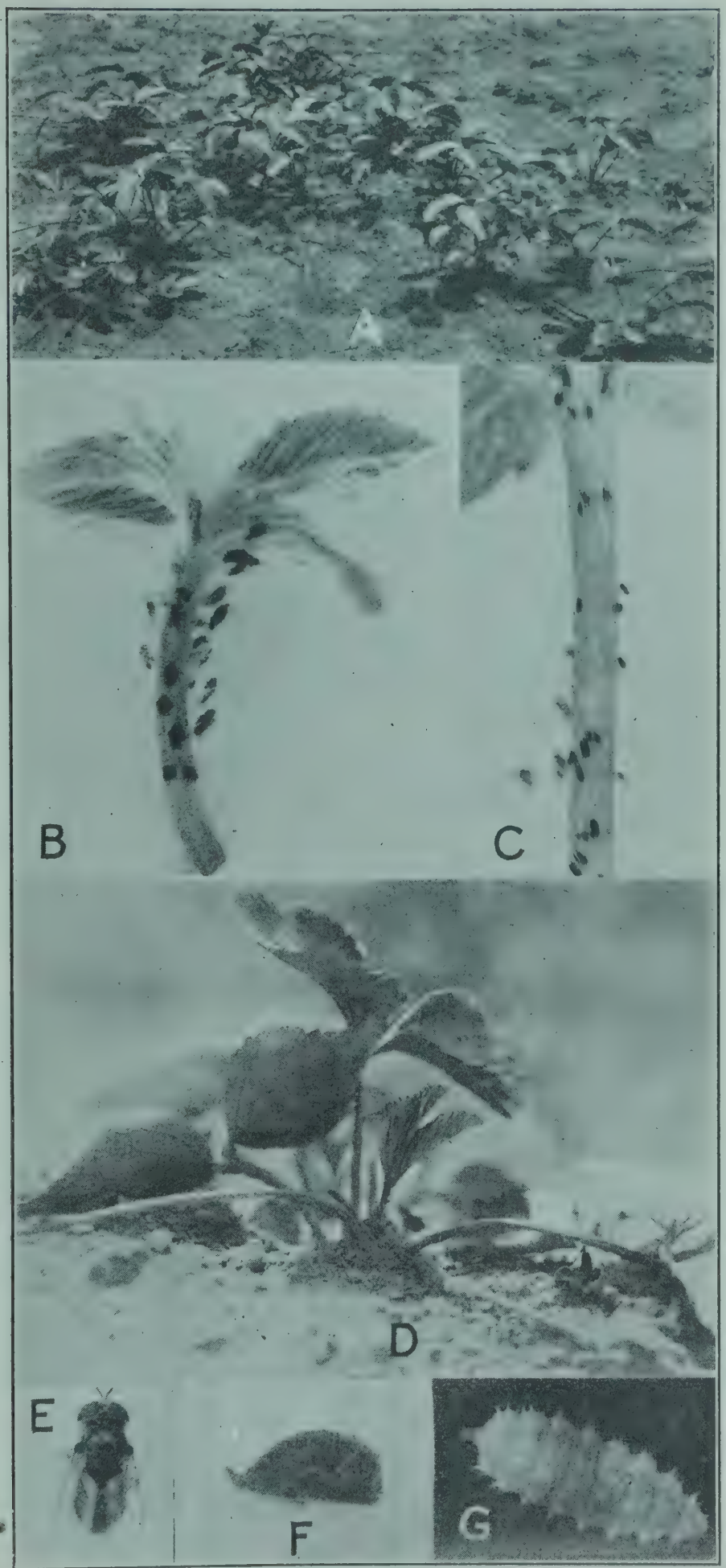
Color characters: The general coloring of the stem-mother is bluish-green, head greenish, with anterior portion lighter. Eyes dark red; antennae with segments 1, 2, and basal portion of 3, pale yellow; remainder of segments dark brown; beak yellowish, tip black, reaching to base of third pair of legs; legs yellowish; tarsi dusky; cornicles yellowish-brown, darker at tip; cauda yellowish brown, clothed with whitish hairs; posterior border of terminal abdominal segments, with a whitish pulverulent stripe; genital plate greenish-yellow; anal plate dark green.

Morphological characters: Body ovate, tending to pear-shape; antennae six-jointed, with segments 3 and 4 not differentiated; thorax with a prothoracic tubercle on each side; cornicles tubular, tapering distally.

Measurements: Length, 1.3 to 1.5 mm.; width, 0.74 to 0.78 mm.; antennae, segment No. 1, 0.05 mm.; segment No. 2, 0.04 mm.; segment No. 3, 0.22 to 0.24 mm.; segment No. 4, 0; segment No. 5, 0.10 to 0.11 mm.; segment No. 6, 0.06 to 0.18 to 0.20 mm.; cornicles, length, 0.19 to 0.21 mm.

EXPLANATORY LEGEND FOR PLATE 1

A.—Mother plant with 18 runners. Photograph made in midsummer from plot artificially infested with *Aphis forbesi*, showing vigorous condition
B.—Oviparous females of *Aphis forbesi* clustered on young tender leaf
C.—Egg of *Aphis forbesi* on pedicel of strawberry leaf
D.—Mound built by *Pheidole vinelandica* around pedicel of strawberry leaf protecting *Aphis forbesi*
E.—*Paragus tibialis:* Female, parasite on *Aphis forbesi*
F.—*Paragus tibialis:* Pupa
G.—*Paragus tibialis:* Larva



(For explanatory legend see p. 442)

WINGED VIVIPAROUS FEMALE (PL. 2, A)

Color characters: The general coloration is black. Head dark brown; eyes dark red, ocelli yellowish; antennae varying from pale yellow to dusky; beak yellowish, darker toward the tip; prothorax black, with anterior and posterior margins greenish, mesothorax and metathorax shining black, suffused with brown on the sides, legs yellowish; tarsi darker; abdomen greenish, with lateral margins of segments 1 to 5 with spotted black; cornicles brownish; cauda and genital plate green; anal plate black.

Morphological characters: Antennae six jointed, joints 3 and 4 sometimes indistinct, third segment with three sensoria; prothorax with two lateral tubercles; abdomen with a very small lateral tubercle; cornicles long, tubular, and flanged at the tip.

Measurements: Length, 1.3 to 1.8 mm., width, 0.7 to 0.8 mm., antennae, length, 0.9 to 1.1 mm.; segment No.1, 0.05 mm.; segment No. 2, 0.05 mm.; segment No. 3, 0.19 to 0.24 mm.; segment No. 4, 0.12 to 0.18 mm.; segment No. 5, 0.13 to 0.16 mm.; segment No. 6, 0.35 to 0.40 mm.; cornicles, length, 0.2 to 0.22 mm.

OVIPAROUS FEMALE (PL. 2, C AND E)

Color characters: General coloring yellowish-green; head yellowish-brown, sometimes greenish on posterior half; antennae with segments 1, 2, and basal portion of 3 pale yellow, remainder of segments darker; eyes dark red; beak greenish at base, pale yellow at middle, and dark at tip; legs pale yellow, tarsi darker; cornicles brownish, darker at tip; cauda dusky, clothed with whitish hairs; margins of abdomen greenish, with center of dorsum yellowish when the abdomen is distended with eggs; posterior abdominal segments more or less covered with a whitish pulverulence; genital plate greenish, with two circular yellowish areas on either side; anal plate dark green.

Morphological characters: The body is more elongate and narrower than in apterous viviparous female, tapering posteriorly. Antennae (pl. 3, H) six-jointed as in viviparous female; thorax with lateral tuberlces on prothorax; hind tibia (pl. 3, I) with about eight sensoria, not at all swollen, and hardly to be distinguished from the other forms; abdomen with a tubercle on either side; cornicles short, tubular, and flanged at the tip (pl. 3, E); genital plate more rounded than in the apterous viviparous female.

Measurements: Length, 1.2 to 1.5 mm.; width, 0.6 to 0.7 mm.; antennae, segment No. 1, 0.05 mm.; segment No. 2, 0.05 mm.; segment No. 3, 0.28 mm.; segment No. 4, 0; segment No. 5, 0.12 mm.; segment No. 6, 0.10 to 0.24 mm.; cornicles: length, 0.12 mm.

MALE (PL. 2, D AND F)

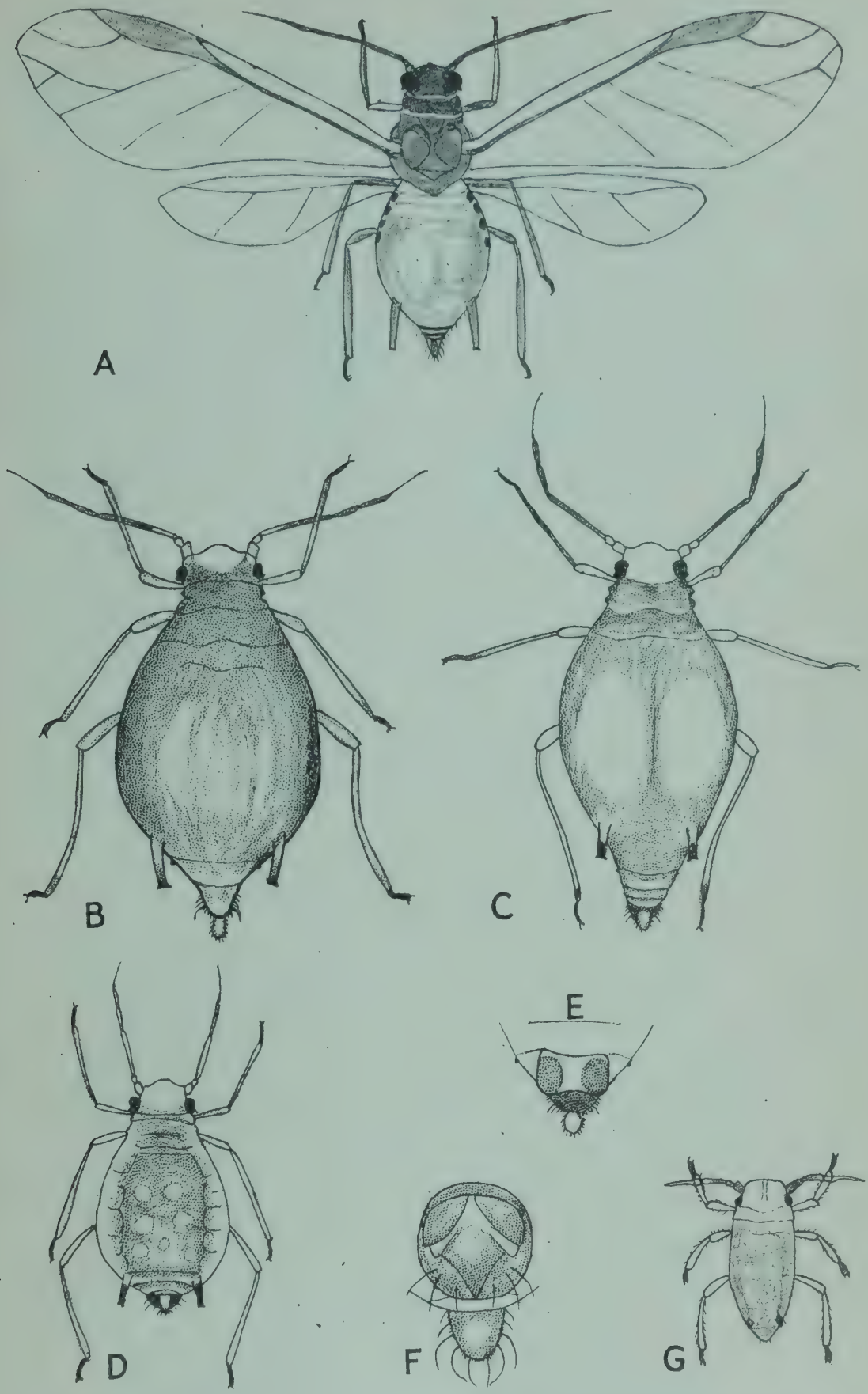
Color characters: General coloration brownish-yellow, mixed with green; antennae brownish-yellow, sensoria darker; head brownish, darker on posterior half; eyes dark brown, beak pale yellow, tip brown, thorax greenish; legs yellowish, with coxae, trochanters, and tarsi darker; cornicles brownish, darker at the tip; abdomen brownish spotted with pale yellow in the center, posterior segments dusky; cauda dusky, clothed with whitish hairs; genital plate brownish; anal plate black.

Morphological characters: Body short and broad; antennae about as long as the width of the body, six-jointed, with five to seven sensoria on segment 3, and one sensoria on each of segments 5 and 6; prothorax with small tubercles; abdomen with lateral margins flattened, a lateral tubercle on each side; cornicles short, tubular, slightly curved, and flanged at the tip; genital plate bears two claspers clothed with spines and hairs.

Measurements: Length, 1 mm.; width, 0.64 mm.; antennae, segment No. 1, 0.04 mm.; segment No. 2, 0.04 mm.; segment No. 3, 0.20 mm.; segment No. 4, 0; segment No. 5, 0.088 mm.; segment No. 6, 0.058+ to 0.18 mm.; cornicles, length, 0.12 mm.

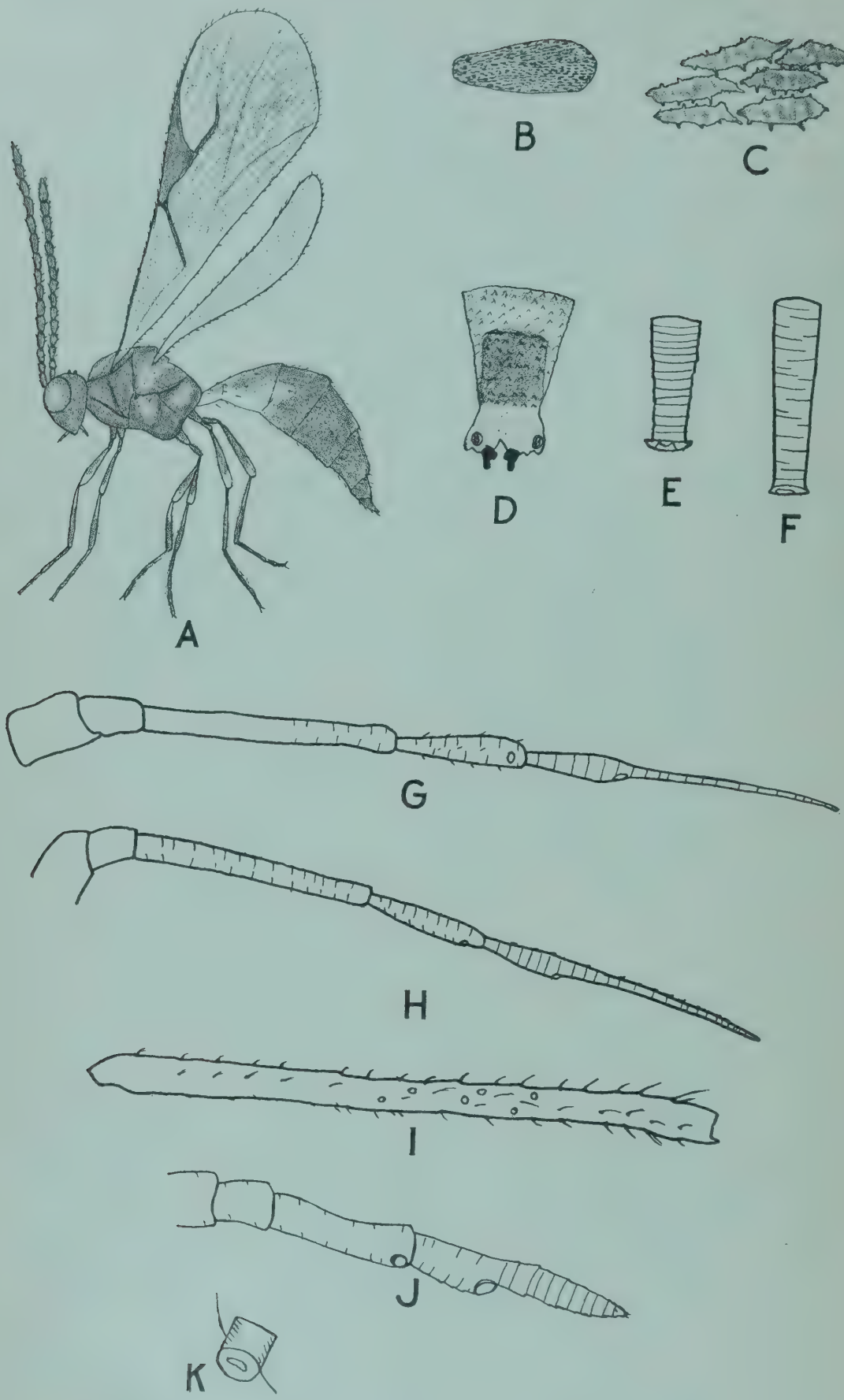
SPRING FORMS

The majority of the second generation of the insect becomes wingless viviparous females (pl. 2, B, and pl. 3, F, G), although some winged ones are often present and distribute the species to other strawberry plants or beds. The young of the second generation are pale yellowish-green in color, in contrast to the dark green of the stem-mothers. The second and succeeding generations are usually found feeding on the pedicels of the young tender leaves, but are often deeply imbedded in the crown of the plant. They are sometimes located on the roots of the plants, but in Tennessee this is rare. Large numbers of plants were pulled to determine the location of the lice, and, except in a few cases, they were present on the pedicels of the young leaves. A generation of lice is produced about every 14 days throughout the summer, with winged ones appearing in each generation after the first.



A.—Winged viviparous female
B.—Wingless viviparous female
C.—Oviparous female
D —Male

E.—Posterior end of venter of oviparous female
F.—Posterior end of venter of male
G.—First instar of stem-mother



(For explanatory legend see p. 447)

In midsummer it is difficult to find any lice at all, owing to the activities of their natural enemies. Both young and mature forms were present on the rootlets as early as April 11.

THE TRUE SEXES

The true males and females make their appearance in Tennessee late in October, and may be found on the plants until February, when the eggs are ready to hatch. After maturing, the females deposit eggs, which carry the species over the winter. Although viviparous females may be present on the plants as late as December, they invariably die and do not assist in the reproduction of the species.

The males are small, wingless, and comparatively few in number. On one leaf pedicel there were counted 2 males and 22 oviparous females. The oviparous females can be readily recognized by their elongate appearance (pl. 1, B) and lighter color, caused by yellowish eggs showing through the chitin. In 1922 the first eggs were laid on November 9, although active egg laying does not begin until December. The eggs when first deposited are orange colored, and it is not until several days later that the characteristic shiny black color is assumed. The number of eggs deposited by a single female varies from four to eight.

FACTORS WHICH INFLUENCE THE APPEARANCE OF THE SEXES

With remarkably few exceptions, the true sexes in plant lice, including *Aphis forbesi*, make their appearance in the North Temperate Zone in the fall of the year and pass the winter in the egg stage. In the more southern localities plant lice generally reproduce throughout the year without the appearance of true sexes or eggs. Therefore, it is natural to conclude that the factor stimulating the formation of true sexes must be temperature; and such was assumed to be the case until it was noticed that the sexes made their appearance somewhat earlier on a plant growing in the laboratory than they did in the field. The laboratory air is warmer than that outside in the latter part of October and November, so that as far as the factor of temperature is concerned there seemed to be no correlation. There is, however, a marked difference between the intensity and duration of daily light exposure in the laboratory and the field. Garner and Allard [1] have shown that the length of daily light exposure influences the flowering and fruiting of plants, and since plant lice and plants both have asexual and sexual stages, which make their appearance at stated intervals, it occurred to the writer that possibly light rather than temperature was the determining factor in the formation of the sexes, and such was found to be the case.

PRODUCTION OF TRUE SEXES AND ITS RELATION TO THE SHORT DAYS OF FALL

One of the familiar phenomena of the Temperate Zone in the autumn is the shortening of the length of day. To create an artificial short day the potted plants used in the experiments were placed out of doors in a dark, ventilated chamber at 5 p. m. and kept there until 9.30 the next morning, when they were removed and placed in the light. The plants and plant lice were thus subjected each day to seven and one-half hours of light exposure. Any response on the part of the insects could hardly be attributed to temperature, since the temperature inside was but 2° to 3° F. higher than that outside. Single potted plants were also darkened by having inverted over them a larger pot or box.

In 1922 the plant with lice was subjected to a short day, beginning May 23. The first males and oviparous females were observed to make their appearance on September 18. Eggs were deposited on September 22, this being about seven weeks earlier than the first eggs are deposited in the field.

[1] GARNAR, W. W., and ALLARD, H. S. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. Jour. Agr. Research 18: 553-606, illus. 1920.

EXPLANATORY LEGEND FOR PLATE 3

A.—*Diaeretus fuscicornis*
B.—*Paragus tibialis:* Egg
C.—*Paragus tibialis:* Egg under high magnification showing sculpturing
D.—*Paragus tibialis:* Caudal appendage
E.—*Aphis forbesi:* Cornicle of oviparous female
F.—*Aphis forbesi:* Cornicle of wingless viviparous female
G.—*Aphis forbesi:* Antenna of wingless viviparous female
H.—*Aphis forbesi:* Antenna of oviparous female.
I.—*Aphis forbesi:* Hind tibia of oviparous female
J.—*Aphis forbesi:* Antenna of first instar stem-mother
K.—*Aphis forbesi:* Cornicle of first instar stem-mother

In 1923 the plants were given a short day, beginning February 23, at which time the eggs were already hatching. By May 7 males and oviparous females had made their appearance, and the first eggs were deposited on May 22. In another experiment, beginning March 21, a plant with lice in the second generation was subjected to a short day, and by May 12 the oviparous females had made their appearance.

THE TRUE SEXES AS INFLUENCED BY A LONG DAY

According to the above experiments the production of the true sexes appears to be governed by the short days of the fall. How, then, would a daily light exposure of 15 hours or more act, and would the production of viviparous forms continue? To determine this point, some strawberry plants infested with lice were placed out of doors September 4 and given 15 hours of daily light exposure by means of a 60-watt electric light, which hung about 2 feet from the plants. At this distance the light did not raise the temperature of the air around the plants, as shown by several tests. A control plant was placed inside the laboratory, next to the window at the same time. On October 6 viviparous females made their appearance on the control plant, but were not yet present on the plants given the long day. On November 5 eggs were found on the control.

When examined on December 22, viviparous forms only were still present out of doors on the plants receiving the long exposure. However, the sexes did make their first appearance on January 4, 1924. This would indicate that a long day may successfully inhibit the formation of the true sexes, provided a certain temperature level is maintained. The first 15 days of December averaged 40° F. and it is very likely that it was in this period that sex formation was initiated. In addition to a certain temperature level, possibly minimum temperatures may be factors. A minimum temperature of 28° on November 9 and 10, and 20° on December 15 was recorded.

Garner and Allard [2] have found that biennials will behave as annuals when subjected to a combination of long days and a lower temperature level. Lower temperatures alone would not accomplish this result.

It should be noted that the strawberry plants bearing aphids were given a long daily light exposure beginning September 4. The experiment was started early enough to insure if possible against the production of the sexes. In 1922, strawberry plants were given a long daily exposure beginning October 12, and a few sexed individuals appeared two weeks later. This shows that once the tendency toward true sex production is initiated, it can not be done away with. However, in the early part of September that tendency is still absent in *Aphis forbesi* in Tennessee.

RELATION OF ANTS TO THE APHIS

Sanderson [3] records that the ant, *Lasius alienus*, attends the strawberry root louse in Delaware and carries it down to the roots of the plants, especially where the soil is sandy. In Tennessee various species of ants have been observed to care for the lice, but the species that is by far mostly commonly found is the little brown ant, *Pheidole vinelandica*, as determined by W. M. Wheeler. This species may be found about strawberry plants in March and up to January. Its method of tending the lice in order to obtain honeydew is interesting. Since the lice are most often clustered on the pedicels of the small, tender leaves, the ants protect them by building small craterlike mounds of dirt about the crown, so that the pedicels of the young leaves may be entirely covered and concealed from view, as shown in Plate 1, D. These little craters are often 2½ inches high, and sometimes completely cover the crown of the plant. In such cases lice may be found feeding on the crown of the strawberry.

NATURAL ENEMIES

The strawberry root louse will sometimes increase in numbers to such an extent that it becomes noticeable, but in most cases its natural enemies keep it in check. Several different species of these were bred, among them a syrphid that had never been reported as preying upon the strawberry root louse, and an undetermined chalcid. Parasites were bred as late as December 14 and as early as May 14. Ladybird beetles, such as *Hippodamia convergens* and *Coccinella novemnotato*, were frequently found upon the root louse, as well as a larva

[2] TAYLOR, W. A. REPORT OF THE CHIEF OF THE BUREAU OF PLANT INDUSTRY. U. S. Dept. Agr. Ann. Rpt. 1922/23: 255–288. 1924.

[3] SANDERSON, E. D. THE STRAWBERRY ROOT LOUSE. Del. Agr. Exp. Sta. Bul. 49: 1–13, illus. 1900.

of one of the Chrysopidae. Sanderson [4] records several hymenopterous parasites, among which are *Lysiphlebus myzi* Ashm., L. *salicaphidis* Ashm., *Lygocerus stigmatatus* Sag., and *Adialatus densleonis* Ashm. A louse attacked by parasites appears plump and greenish in color. As the parasite grows the body of the louse becomes much distended, and upon maturity nothing is left of the host except the straw-colored chitinous envelope.

DIARETUS FUSCICORNIS ASHM.

This little braconid (pl. 3, A) as determined by A. B. Gahan, was frequently bred from lice clustered on the pedicels of the leaves, and undoubtedly helps to keep the lice in check. In color, it is shining black, legs yellowish, mixed with brown. Antennae of male 15-jointed, of female 13-jointed. Length 1.6 mm.

PARAGUS TIBIALIS

This syrphid (pl. 1, E, F, G and pl. 3, B, C, D) appears to be the most constant and efficient enemy of the strawberry root louse in Tennessee. The larvae were found in the field as early as April 18. A larva was observed to devour seven lice in five minutes in the laboratory. Larvae collected on May 20 pupated May 22, and the adults emerged May 29. The puparium is inconspicuous on the under side of the leaves, and rather resembles a piece of dirt.

EGG.—Chalk-white in color. In outline, subcylindrical and ovate; truncated at the narrow end and rounded at the other. The surface of the egg is covered with very fine microscopic elevations arranged in lines, which under high magnification are seen to possess projecting arms, as shown in pl. 3, C. Length, 1 mm.; width, 0.40 mm.

LARVA.—General coloring pale yellow, mixed with patches of brown; posterior respiratory appendages dark brown at base, yellowish on apical third. Dorsal spiracular spine brownish. Surface of skin covered with minute, wartlike elevations. The spines on segment 4 appear equal. Beyond this segment the dorso-lateral spines are the longest, the dorsal spines small. Dorsal spiracular spine large, concave lateral, bifurcate. Length, 6 to 7 mm. Length of posterior respiratory appendage, 0.46 mm.

ADULT.—General coloring black, frequently reddish on the abdomen. Both male and female with a median black band on the face. Length, 3 to 5 mm.

SUMMARY

Since strawberries are classed as nursery stock in some States and required to be inspected for the strawberry root louse, *Aphis forbesi* Weed, it becomes necessary to determine more exactly its economic importance. Observation and experiments since 1919 show that *Aphis forbesi* is present in all the strawberry-growing areas of Tennessee and that it can not be classed as highly injurious in that State. It is not a leaf curler and apparently injects no toxin into the host plant.

Plants were artificially infested with the root louse and kept so throughout the summer to determine the effects on the production of runners. These plants each produced an average of 47.6 plants as compared with 49.7 plants produced by the controls.

Aphis forbesi passes the winter in the egg stage on the pedicels of the leaves. Hatching begins about February 15 and continues for about a month. Reproduction continues viviparously throughout the summer and as late as November. In Tennessee the lice remain mostly on the pedicels of the leaves and around the crown of the plant. Only rarely are they found on the roots. The ant, *Pheidole vinelandica*, is most commonly associated with *Aphis forbesi*.

The true sexes first make their appearance in October and may be found on the plants until February.

In a study of the factors influencing the production of the true sexes in *A. forbesi*, it was found that the relative length of exposure to daily light appears to be an important factor. By giving a daily short exposure of seven and one-half hours, beginning February 23, the sexes made their appearance on May 7. Eggs were deposited on May 22.

During the fall months of 1923 when the plants were given a long daily light exposure of 15 hours out of doors, the aphids continued to reproduce viviparously as late as January 4, 1924, when the first true sexes made their appearance. On the control plant the sexes appeared October 6. It is possible that a combination of long days and a temperature of 50° F. or higher might have sufficed to keep the sexes from appearing.

The strawberry root louse has many natural enemies, among the most important of which is the little syrphid known as *Paragus tibialis*. Other natural enemies include several species of Braconidae, the most common being *Diaeretus fuscicornis*. Several Coccinelidae and Chrysopidae may frequently be found preying on the lice.

[4] SANDERSON, E. D. REPORT OF THE ENTOMOLOGIST. The strawberry root louse. Del. Agr. Exp. Sta. Ann. Rpt. (1899/1900) 12: 143–169, illus. 1901.

THE EFFECT OF SULPHUR AND GYPSUM ON THE FERTILITY ELEMENTS OF PALOUSE SILT LOAM[1]

By LEWIS W. ERDMAN[2]
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INTRODUCTION

During the past few years some soils have been found to be quite low in total sulphur, and the use of sulphur as a fertilizer has been suggested as a means of remedying this condition. Most of the experiments with sulphur have had certain objects in view: (1) To study its effect on the growth and composition of crops; (2) to determine its effectiveness when inoculated with sulphur-oxidizing bacteria and composted with manure and rock phosphate in rendering available the phosphorus in the rock phosphate; and (3) to determine its effectiveness when composted with manure and greensand, in making the potassium in the greensand available for crops. Very little attention has been given to the direct effect of sulphur on the fertility elements in the soil. From the soil fertility point of view, this problem is of unusual significance, because the future value of sulphur as a fertilizer will depend not only upon its ability to increase crop growth, but also upon the reactions which it brings about in the soil, and especially upon its effects on those elements which have a direct bearing on fertility.

The present investigation was planned to ascertain to what extent the sulphur, potassium, phosphorus, nitrogen, calcium, and magnesium of the Palouse silt loam, an important soil type in Washington, are affected by applications of uninoculated sulphur, inoculated sulphur, and gypsum.

EXPERIMENTAL WORK

The soil used was a typical Palouse silt loam secured from an alfalfa pasture on the experimental farm of the State College of Washington. Wheat is grown extensively on this particular soil. It is derived mainly from basaltic rock and, judging from the following chemical analysis, may be considered of good fertility.

Pounds per 2,000,000 pounds of soil: Nitrogen, 2,900; phosphorus, 1,280; potassium, 42,400; sulphur, 960; calcium, 29,200; magnesium, 20,200.

Twenty-four ordinary clay flowerpots (inside diameter at top 12 inches, at bottom 8.75 inches, and depth 8.5 inches) were used for this work. These were divided into two series of 12 each, the first series being used for experiment 1, and the other for experiment 2. Each pot was thoroughly paraffined on the inside, and in the bottom was placed a paraffined cork stopper through which a short glass tube was inserted to permit the removal of drainage water. The outlet of the tube was covered with a very small inverted clay pot to prevent the soil from clogging the opening.

EXPERIMENT 1

In an open field on the college farm a stand was built large enough to hold 12 pots and just high enough to allow 2.25-liter acid bottles to be placed beneath. The glass tube leading from each pot entered one of these bottles, the mouth of which was covered to exclude rain and dust particles. On November 27, 1922, 18½ lbs. of soil (moisture-free basis) was placed in each pot. This amount of soil approximated the ordinary depth of surface soil (6⅔ inches). The following treatments, made in duplicate, were thoroughly mixed in the upper 2 inches of soil on December 5, 1922.

[1] Received for publication June 17, 1924; issued May, 1925. Published with the approval of the director of the Washington Agricultural Experiment Station as Scientific Paper No. 109. The work reported in this paper was done in the laboratories of the Division of Chemistry of the Washington station under the direction of Dr. J. R. Neller.

[2] Formerly research fellow, National Research Council Sulphur Fellowship. Now assistant professor of soils and assistant chief in soil bacteriology, Iowa State College. The writer wishes to acknowledge his indebtedness to the Sulphur Fellowship Committee of the National Research Council for the appointment to this fellowship; to Dr. J. R. Neller for many helpful suggestions; and to Dr. P. E. Brown for reading the manuscript.

Pot No.	Treatment	Pounds per acre
1 and 2	Control	
3 and 4	Uninoculated sulphur	186
5 and 6	Inoculated sulphur	189
7 and 8	Uninoculated sulphur	1,000
9 and 10	Inoculated sulphur	1,015
11 and 12	Gypsum	1,000

The soils in pots Nos. 3, 4, 5, 6, 11, and 12 received equivalent amounts of sulphur, the calculations being made on the basis that chemically pure gypsum, which was used in these experiments, contained 18.6 per cent of sulphur. The samples of uninoculated and inoculated sulphur were obtained from the Texas Gulf Sulphur Co. Inoculated sulphur is supposed to contain large numbers of efficient sulphur-oxidizing bacteria which give it the ability to act quicker than uninoculated sulphur.

The soils in the pots placed in the field were exposed to natural weather conditions and remained undisturbed from December 5, 1922, to June 18, 1923. On the latter date a composite soil sample was taken from each pot, and water extractions were made for the purpose of studying the amount of water-soluble plant food material in the soils at the close of the experiment. During the progress of the experiment the drainage water from each pot for each month was collected, filtered through Buchner funnels, and stored in large bottles in the laboratory. A small quantity of toluol was added to each bottle to inhibit bacterial action.

EXPERIMENT 2

On December 9, 1922, an exact duplication of experiment 1 was started in the greenhouse to verify and elaborate the results obtained from the soils exposed to natural weather conditions. After the treatments were made the soils were adjusted to the optimum moisture content, and for a period of 72 days the moisture content was maintained by additions of distilled water to weight. At the end of this time all of the soils were flooded with definite amounts of distilled water for 4 consecutive days, and the leachings thus obtained were treated and analyzed as in the case of the drainage water secured from the pots in experiment 1. Soil extracts wese made from these soils after they were allowed to dry.

ANALYTICAL WORK

The analytical work for these experiments involved (1) the analyses of the drainage water obtained form the pots which were exposed to field conditions; (2) the analyses of the leachings from the pots which were placed in the greenhouse; and (3) the analyses of the water extracts made from the soils at the close of both experiments. These analyses included determinations of sulphur, nitrogen, phosphorous, potassium, calcium, and magnesium. Colorimetric hydrogen-ion determinations were made on samples of soil taken from the pots at the conclusion of both experiments and also on samples of the drainage water obtained from each pot for the month of June, 1923.

A rain gauge of the standard United States Weather Bureau type was placed beside the pots used in experiment 1. Records were kept of the amount of precipitation, and samples of rain water for each month were analyzed for total sulphur. A few ammonia and nitrate determinations were made on the first samples, but since only traces of these compounds were present the study of nitrogen in the rain water was abandoned.

DISCUSSION OF RESULTS

This study was limited to the surface 6⅔ inches of soil, and it should be understood that the entire amount of elements reported in the drainage water was not necessarily totally lost to crops. Under actual field conditions some of the water-soluble elements may be returned to the surface soil from the deeper soil layers by capillarity. It is believed, however, that the results secured are sufficient to aid materially in obtaining a better understanding of the probable effects of sulphur on soil fertility.

Climatic conditions at the Washington Agricultural Experiment Station are ideal for lysimeter studies. The annual average rainfall is 21.49 inches. The heavy rains, when leaching is most apt to occur, come during the period extending from November to June. The season during which these experiments were made was exceptionally dry in the fall, and the winter rains did not begin until early in December, or immediately after the pots were placed in the field. Therefore as far as the percolation studies are concerned this investigation may be considered as representing the work of one year.

The rainfall and the total sulphur of the rain and snow covering the period from November 29, 1922, to June 18, 1923, are found in Table I.

TABLE I.—*Amount of rainfall, and sulphur in rain and snow from November 29, 1922, to June 18, 1923*

Date	Amount of rainfall	Pounds per acre	
		Sulphur	Calculated as SO_3 sulphur
	Inches		
1922. No. and Dec.	4.18	0.83	1.87
1923. Jan.	3.75	1.11	2.77
Feb.	.95	.54	1.35
Mar.	.54	.48	1.20
Apr.	.88	.29	.72
May	1.30	.70	1.75
June	3.25	----------	----------
Total	14.85	3.95	9.66

The total amount of rainfall for the eight months was 14.85 inches, which indicates that the rainy season for 1922 to 1923 was not quite up to the average. For this reason the results obtained from this study are probably all the more valuable. The total amount of sulphur added to an acre of soil in the rain and snow for this period (exclusive of the June sample which was contaminated by birds) was 3.95 lbs. If 2 lbs. of sulphur is allowed for the months of June and October, which seems to be a fair estimate, judging from the other analyses this would make a total of about 6 lbs. of sulphur per acre reaching the soil through the annual precipitation. This quantity is very much below that reported by some other investigators. The writer [3] found an average of about 15 lbs. of sulphur added to an acre of soil in Iowa by the rain water. Other analyses of the rain falling in cities and towns vary from 25 to over 300 lbs. of sulphur per acre annually. The results obtained for the Palouse country are probably low, because this section is far removed from the great coal-consuming centers which furnish the atmosphere with the greater portion of its sulphur.

Table II shows the amount of drainage water and leachings collected from the pots in both experiments.

TABLE II.—*Amount of drainage and leachings*

Pot No	Treatment (pounds per acre)	Experiment 1 (field pots)				Experiment 2 (greenhouse pots), total leachings
		December	January and February	March and June	Total for five months	
		C.c.	*C.c.*	*C.c.*	*C.c.*	*C.c.*
1	Control	1,930	5,835	2,655	10,420	6,555
2	do	1,869	4,515	2,640	9,024	6,945
	Average	1,900	5,175	2,648	9,722	6,750
3	Uninoculated sulphur 186	2,329	6,965	3,215	12,509	7,300
4	do	2,245	5,200	2,875	10,320	6,460
	Average	2,287	6,083	3,045	11,415	6,880
5	Inoculated sulphur 189	2,305	5,960	3,210	11,475	7,090
6	do	1,904	5,250	2,870	10,024	6,705
	Average	2,105	5,605	3,040	10,750	6,898
7	Uninoculated sulphur 1000	2,379	6,690	3,180	12,249	6,725
8	do	2,057	4,095	2,660	8,812	6,610
	Average	2,218	5,393	2,920	10,531	6,668
9	Inoculated sulphur 1015	2,269	5,380	2,960	10,609	6,430
10	do	2,107	4,937	2,765	9,809	6,065
	Average	2,188	5,159	2,863	10,209	6,248
11	Gypsum 1000	2,289	5,515	2,800	10,604	6,580
12	do	2,127	5,167	2,725	10,019	6,630
	Average	2,208	5,341	2,763	10,312	6,605

[3] ERDMAN, L. W. THE SULPHUR CONTENT OF RAINWATER. Soil Sci. 14: 363–367. 1922.

A striking uniformity is observed in the amount of drainage water leached through each pot. The small discrepancies may readily be accounted for by the fact that the bottoms of some of the clay pots were slightly raised in the center. This would allow a certain amount of water to accumulate and remain in these pots after each rain. The drainage water for January and February was combined for analysis, and likewise the March drainage was added to the June drainage water. Owing to the small rainfall for April and May there was no percolation of water through the pots for these months.

SULPHUR IN DRAINAGE WATER AND LEACHINGS

It is not known what proportion of the total sulphur in the soil is present in the organic form and how much is free or combined with the soil minerals. In soils well supplied with organic matter probably a large portion of the sulphur is organic, because sulphur forms part of the protein molecule and is therefore present in all plants and animals. The decomposition of organic sulphur compounds results in the formation of thiosulphates, hydrogen sulphide, and free sulphur, and all of these substances may be oxidized to sulphates by the sulphur-oxidizing microorganisms. This process, termed sulphofication, is probably responsible for the large losses of sulphur in the drainage water. Lyon and Bizzell [4] have tabulated the results of a number of investigators and found that the loss of sulphur in drainage water varied from 8 to 281 lbs. per acre, depending upon the type of soil and the kind of cropping system followed.

By applying elemental sulphur to soils and measuring the sulphates coming through in the drainage water, a definite idea of the rate and extent of sulphofication may be determined. In Table III are found the results of the sulphur analyses of the drainage water and leachings obtained from the variously treated soils in both experiments.

TABLE III.—*Amount of sulphur in drainage water, leachings, and soil extracts*

Pot No.	Treatment (pounds per acre)	Experiment 1 (field pots)—sulphur in pounds per 2,000,000 pounds of soil						Experiment 2 (greenhouse pots)—sulphur in pounds per 2,000,000 pounds of soil		
		December	January and February	March and June	Total for five months	Soil extracts	Total water-soluble sulphur	Leachings	Soil extracts	Total water-soluble sulphur
1	Control	11.7	14.5	21.1	47.3	29.0	76.3	33.2	10.9	44.1
2	do	8.5	4.7	22.2	35.4	22.0	57.4	41.3	12.0	53.3
	Average	10.1	9.6	21.6	41.3	25.9	66.8	37.2	11.4	48.7
3	Uninoculated sulphur 186	9.8	11.1	66.9	87.8	141.6	229.4	151.5	108.2	259.7
4	do	11.3	13.1	106.9	131.3	156.5	287.8	200.7	80.4	281.1
	Average	10.5	12.1	86.9	109.5	149.0	258.6	176.1	94.3	270.4
5	Inoculated sulphur 189	11.2	10.2	77.5	98.9	138.9	237.8	169.9	114.3	284.2
6	do	9.4	10.4	102.0	121.8	163.9	285.7	138.1	87.2	225.3
	Average	10.3	10.3	89.7	110.3	151.4	261.7	154.0	100.7	254.7
7	Uninoculated sulphur 1000	8.7	9.8	156.9	175.4	416.1	591.5	544.6	746.5	1,291.1
8	do	9.3	17.8	146.3	173.4	456.6	630.0	515.6	649.5	1,165.1
	Average	9.0	13.8	151.6	174.4	436.3	610.7	530.1	698.0	1,228.1
9	Inoculated sulphur 1015	13.2	13.4	182.5	209.1	483.5	692.6	552.4	711.1	1,263.5
10	do	10.1	14.0	154.5	178.6	472.0	650.6	529.8	598.4	1,128.2
	Average	11.6	13.7	168.5	193.8	477.7	671.6	541.1	654.7	1,195.8
11	Gypsum 1000	100.2	169.1	30.5	299.8	47.2	347.0	98.6	69.9	168.5
12	do	104.8	180.4	30.3	315.5	35.0	350.5	178.4	65.4	243.8
	Average	102.5	174.7	30.4	307.6	41.1	348.7	138.5	67.6	206.1

[4] LYON, T. L., and BIZZELL, J. A. LYSIMETER EXPERIMENTS. RECORDS FOR TANKS 1 TO 12 DURING THE YEARS 1910 TO 1914 INCLUSIVE. N. Y. Cornell Agr. Exp. Sta. Mem. 12, 115 p., illus. 1918.

These data reveal some interesting facts. Considering first, the results of experiment 1, it may be noticed that after three months there was only a slight oxidation of the elemental sulphur, owing possibly to the cold temperatures prevailing during this time. From February to June the temperature was more favorable for the activity of soil organisms, and a considerable portion of the elemental sulphur from both the small and large applications was changed to the sulphate form. Practically the same amount of sulphates was produced from the small applications of uninoculated and inoculated sulphur. Slightly more sulphates were formed from the large application of inoculated sulphur than from the same amount of uninoculated sulphur.

It was realized that the amount of rainfall percolating through the soil in the pots was not sufficient to remove all of the sulphur which had been changed to sulphates. Therefore, at the conclusion of the experiments, soil extracts were made from a composite soil sample from each pot in order to throw more light on the changes that the elemental sulphur had undergone in the soil. The data obtained from these extracts were added to the amount of sulphur recovered in the drainage in order to get the total water-soluble sulphur. By examining the latter data it is noted, first, that after seven months approximately 200 lbs. of sulphate sulphur was obtained from the low-sulphur pots Nos. 3, 4, 5, and 6, and, second, that a little more than one-half of the 1,000 pounds of sulphur added to pots Nos. 7, 8, 9 and 10 was recovered as sulphates.

These facts indicate that this soil has a very marked sulphofying power, which probably explains why the inoculated and uninoculated sulphur treatments produced almost the same quantity of sulphates.

It may be well to recall that the soil in the pots in the greenhouse was maintained at optimum moisture conditions for a period of 72 days. During this time conditions should have been extremely favorable for optimum microorganic activity, and the data given for experiment 2 show that such conditions did exist. The results for the small sulphur treatments agree almost perfectly with the data obtained from experiment 1. In the case of the larger sulphur additions it is surprising to note that the total 1,000 lbs. of sulphur which was originally added was recovered as sulphates after two and one-half months. The large sulphur treatments seemed to have had a marked influence also on sulphofication, as is shown by the increase in sulphur obtained over the amount added and the amount recovered from the untreated soils. These data also show that for this soil the uninoculated sulphur is just as effective as the inoculated sulphur in producing available sulphur for plants.

A striking feature of the December analysis is that practically one-half of the sulphur added in the 1,000 lbs. of gypsum leached through the surface soil at the end of one month. After three months all of the sulphur originally added, and an appreciable quantity besides, appeared in the drainage water. By June the gypsum-treated soil showed a still further increase in sulphur in the drainage water when compared with the control. These results would seem to indicate that gypsum greatly increased sulphofication in this soil. The results obtained in the greenhouse for the soil treated with gypsum, however, do not agree with these findings, but show on the other hand that gypsum did not increase sulphofication. The data obtained from the field pots treated with gypsum present a strong argument for the spring application of gypsum, because if applied in the fall it may be largely lost through leaching before the crop has a chance to make use of it.

EFFECT OF SULPHUR AND GYPSUM ON POTASSIUM

The results of the analyses of the drainage water and leachings for potassium are given in Table IV.

An examination of the amount of potassium leached from the control soil indicates that the Palouse silt loam is not deficient in available potassium. All of the sulphur treatments caused small but decided increases in the amount of potassium appearing in the drainage water. The results of both experiments confirm this statement, although it is not known why so much more water-soluble potassium was present in the drainage water and water extracts from the field soils than was the case in the soils in the greenhouse. The process of making potassium available is certainly correlated with sulphofication, because, as shown by the analyses of the drainage water obtained during the different months, no effect on soil potassium was produced until the end of June, or after appreciable quantities of sulphur had been oxidized. This statement is very

TABLE IV.—*Amount of potassium in drainage water, leachings, and soil extracts*

Pot No.	Treatment (pounds per acre)	Experiment 1 (field pots)—potassium in pounds per 2,000,000 pounds of soil						Experiment 2 (greenhouse pots)—potassium in pounds per 2,000,000 pounds of soil		
		December	January and February	March and June	Total for five months	Soil extracts	Total water-soluble potassium	Leachings	Soil extracts	Total water-soluble potassium
1	Control	24.1	35.9	120.8	180.8	244.4	425.2	27.5	156.3	183.8
2	do	25.0	22.9	202.7	250.6	231.5	482.1	56.1	157.2	213.3
	Average	24.5	29.4	161.7	215.7	237.9	453.6	41.8	156.7	198.5
3	Uninoculated sulphur 186	26.8	32.8	160.7	220.3	278.1	498.4	80.0	176.0	256.0
4	do	24.0	25.4	195.8	245.2	262.1	507.3	82.5	169.8	252.3
	Average	25.4	29.1	178.2	232.7	270.1	502.8	81.2	172.9	254.1
5	Inoculated sulphur 189	25.0	27.1	181.0	233.1	254.0	487.1	75.6	182.4	258.0
6	do	21.6	24.5	181.1	227.2	242.8	470.0	74.5	171.2	245.7
	Average	23.3	25.8	181.0	230.1	248.4	478.5	75.0	176.8	251.8
7	Uninoculated sulphur 1000	25.7	31.9	215.9	273.5	302.2	575.7	131.3	226.7	358.0
8	do	25.3	23.5	227.9	273.5	287.8	561.3	131.9	201.6	333.5
	Average	25.5	27.7	221.9	275.1	295.0	568.5	131.6	214.1	345.7
9	Inoculated sulphur 1015	28.7	24.5	268.1	321.3	285.4	606.7	131.4	234.4	365.8
10	do	26.3	31.4	235.3	293.0	298.2	591.2	113.4	200.2	313.6
	Average	27.5	27.9	251.7	307.1	291.8	598.9	122.4	217.3	339.7
11	Gypsum 1000	38.7	54.6	106.9	200.2	241.1	441.3	60.8	172.2	233.0
12	do	41.1	58.9	114.1	214.1	233.1	447.2	84.6	183.3	267.9
	Average	39.9	56.7	110.5	207.1	237.1	444.2	72.7	177.7	250.4

strikingly brought out by the data given for the high sulphur treatments. In this connection Ames [5] suggested that the liberation of potassium was brought about by salts formed rather than by the direct action of acidity, developed from nitrification and sulphofication, on insoluble potassium compounds. That this assumption holds true for these experiments will be shown later, when the hydrogen-ion concentrations of the soils are considered.

The inoculated and uninoculated sulphur treatments showed practically the same effect in liberating the insoluble soil potassium.

The data given for the analyses of the drainage water from the soils treated with gypsum indicate that for the first three months gypsum caused an increase in water-soluble potassium, but, on the other hand, the data for the total water-soluble potassium show that gypsum slightly decreased the amount of potassium going into solution. A correct interpretation of the effect of gypsum on soil potassium in experiment 1 is made impossible by the seemingly high analysis reported for the control soil No. 2 for the March and June drainage. The data given for experiment 2 show that under greenhouse conditions gypsum brought about a distinct increase in the amount of potassium found in the leachings and also in the soil extracts.

EFFECT OF SULPHUR AND GYPSUM ON CALCIUM

Tottingham and Hart [6] summed up their work on sulphur and sulphur composts with the statement that "adequate consideration of the use of sulphur as a fertilizer must recognize its tendency to deplete the stock of

[5] AMES, J. W. SOLVENT ACTION OF NITRIFICATION AND SULFOFICATION. Ohio Agr. Exp. Sta. Bul. 351, p. 221–257. 1921.

[6] TOTTINGHAM, W. E., and HART, E. B. SULFUR AND SULFUR COMPOSTS IN RELATION TO PLANT NUTRITION. Soil Sci. 11: 49–65, illus. 1921.

CaO, P_2O_5, and other soil constituents." The extent to which the supply of calcium in Palouse silt loam may be depleted by the use of sulphur is brought out in the data presented in Table V.

TABLE V.—*Amount of calcium in drainage water, leachings, and soil extracts*

Pot No.	Treatment (pounds per acre)	Experiment 1 (field pots)—calcium in pounds per 2,000,000 pounds of soil						Experiment 2 (greenhouse pot)—calcium in pounds per 2,000,000 pounds of soil		
		December	January and February	March and June	Total for five months	Soil extracts	Total water-soluble calcium	Leachings	Soil extracts	Total water-soluble calcium
1	Control	52.9	43.9	36.1	132.9	625.7	758.6	56.4	556.4	612.8
2	do	41.8	32.4	62.7	136.9	487.3	624.2	78.6	574.9	653.5
	Average	47.3	38.1	49.4	134.9	556.5	691.4	67.5	565.6	633.1
3	Uninoculated sulphur 186	44.0	41.4	56.3	141.7	547.2	688.9	138.2	579.8	718.0
4	do	44.7	33.2	89.5	167.4	495.6	663.0	176.0	552.6	728.6
	Average	44.3	37.3	72.9	154.5	521.4	675.9	157.1	566.2	723.3
5	Inoculated sulphur 189	46.1	36.9	68.3	151.3	543.1	694.4	150.0	635.6	785.6
6	do	39.9	30.5	91.7	162.1	526.6	688.7	126.3	552.6	678.9
	Average	43.0	33.7	80.0	156.7	534.8	691.5	138.1	594.1	732.2
7	Uninoculated sulphur 1000	44.8	38.2	129.4	212.4	404.7	617.1	458.2	646.7	1,104.9
8	do	42.8	30.2	94.2	167.2	353.1	520.3	432.8	548.9	981.7
	Average	43.8	34.2	111.8	189.8	378.9	568.7	445.5	597.8	1,043.3
9	Inoculated sulphur 1015	61.1	36.2	163.7	261.0	351.1	612.1	455.8	557.5	1,013.3
10	do	46.3	35.0	103.9	185.2	417.1	602.3	421.0	495.6	916.6
	Average	53.7	35.6	133.8	223.1	384.1	607.2	438.4	526.5	964.9
11	Gypsum 1000	107.6	141.0	34.4	283.0	561.7	844.7	90.6	631.9	722.5
12	do	112.8	152.2	33.9	298.9	541.1	840.0	328.4	646.7	975.1
	Average	110.2	146.6	34.1	290.9	551.4	842.3	209.5	639.3	848.8

For three months the amount of calcium appearing in the drainage water collected from the field pots was practically the same for all of the sulphur treatments. This, however, should be expected, because up to this time no appreciable sulphofication had taken place. As the sulphur was changed to sulphate it might be expected that some of the sulphuric acid would react with the calcium in the soil. This is demonstrated by the amount of calcium appearing in the March and June drainage from the soils treated with sulphur. It is difficult to explain why the high sulphur treatments caused such small amounts of calcium to be present in the soil extracts. The determinations for the duplicate control soils show a wide variation which tend to throw a shadow of doubt on the accuracy of all of the calcium data obtained from the soil extracts in experiment 1.

The results obtained from experiment 2 show that all of the sulphur treatments caused a marked loss of calcium in the leachings. This is especially true for the high sulphur additions. Under the conditions of the greenhouse experiment the data secured support the belief that sulphur rapidly depletes the supply of calcium in the soil. Further experimentation is needed, however, to confirm this statement for field conditions.

Working with various lime compounds, including calcium sulphate, Broughton [7] reached the conclusion that there is nothing in the soil to attract calcium in the form of the sulphate because $CaSO_4$ moves through the different depths in a solid column.

[7] BROUGHTON, L. B. HOW LIME IS DISTRIBUTED THROUGH AND LOST FROM SOILS. FACTORS INFLUENCING THE DIFFUSION AND DEPLETION OF LIME IN SOILS. Md. Agr. Exp. Sta. Bul. 166, p. 285–334. 1912.

If this were true, gypsum would not have any chemical action on the soil constituents. But it is evident from the discussion of the data obtained from experiment 2 that gypsum caused an increase in the amount of potassium in the leachings. Again, it is difficult to interpret the calcium data secured from the soils treated with gypsum in both experiments, because, first, of the wide variation in the calcium determinations in the water extracts from the two control soils in experiment 1 and, second, because of the wide difference between the duplicate gypsum treatments in the amount of calcium found in the leachings in the water-soluble magnesium in the soils at the close of the experiments.

Inasmuch as the differences between duplicate treatments in most cases are greater than the difference noted for the various sulphur treatments, it seems best to discuss only the total water-soluble magnesium data for the two experiments. Considering first the total water-soluble magnesium for experiment 1, it will be noted that none of the treatments had any appreciable effect on this element. The data from

TABLE VI.—*Amount of magnesium in drainage water, leachings, and soil extracts*

Pot No.	Treatment (pounds per acre)	Experiment 1 (field pots)—magnesium in pounds per 2,000,000 pounds of soil						Experiment 2 (greenhouse pots)—magnesium in pounds per 2,000,000 pounds of soil		
		December	January and February	March and June	Total for five months	Soil extracts	Total water-soluble magnesium	Leachings	Soil extracts	Total water-soluble magnesium
1	Control	15.0	10.7	45.0	70.7	205.2	275.9	19.2	Lost.	----
2	do	13.4	5.9	20.6	39.9	189.6	229.5	23.5	187.4	208.7
	Average	14.2	8.3	32.8	55.3	197.4	252.7	21.3	187.4	208.7
3	Uninoculated sulphur 186	13.7	13.2	15.7	42.6	161.8	204.4	34.9	104.8	139.7
4	do	13.3	10.1	23.9	47.3	182.6	229.9	50.3	167.1	217.4
	Average	13.5	11.6	19.8	44.9	172.2	217.1	42.6	135.9	178.5
5	Inoculated sulphur 189	14.0	5.6	19.3	38.9	168.7	207.6	52.5	174.9	227.4
6	do	13.4	6.8	27.2	47.4	167.0	214.4	31.7	155.9	187.6
	Average	13.7	6.2	23.2	43.1	167.8	211.0	42.1	165.4	207.5
7	Uninoculated sulphur 1000	14.5	7.3	36.6	58.4	172.2	230.6	128.6	156.6	285.2
8	do	14.1	6.7	34.2	55.0	142.6	197.6	122.5	130.4	252.9
	Average	14.3	7.0	35.4	56.7	157.4	214.1	125.5	143.5	269.0
9	Inoculated sulphur 1015	19.1	8.8	45.7	73.6	93.9	167.5	136.7	137.6	274.3
10	do	14.7	10.2	35.4	60.3	168.7	229.0	132.1	123.8	255.9
	Average	16.9	9.5	40.5	66.9	131.3	198.2	134.4	130.7	265.1
11	Gypsum 1000	31.9	39.5	10.9	82.3	158.3	240.6	26.8	148.0	174.8
12	do	32.3	44.5	10.7	87.5	147.8	235.3	47.2	116.6	163.8
	Average	32.1	42.0	10.8	84.9	153.0	237.9	37.0	132.3	169.3

experiment 2. The results do indicate, however, that at least the greater portion of the calcium in the gypsum is leached away in the drainage water.

EFFECT OF SULPHUR AND GYPSUM ON MAGNESIUM

In Table VI appear the data showing the losses of magnesium in the drainage water and leachings and experiment 2 show that, while the low sulphur additions did not increase the amount of soluble magnesium, the high sulphur treatments did cause an increase in the amount of magnesium going into solution. In this case some of the soil magnesium was probably used to neutralize some of the acid which was produced in the oxidation of the 1,000 lbs. of elemental sulphur. Gypsum apparently had little effect in liberating magnesium from its insoluble compounds in the soil.

EFFECT OF SULPHUR AND GYPSUM ON SOIL NITROGEN

The data showing the effect of sulphur and gypsum on nitrogen in the soil and on nitrification are found in Table VII.

It should be remembered that the nitrogen which leached through the soils during December represents the accumulation of nitrates during the summer and fall of 1922, for there had been no rains sufficient to leach the soil previous to this time. No effect was noticed for the treatments during December. All of the remaining data indicate that the different treatments caused a slight depressing effect on nitrification. It is difficult to account for the relatively small amount of nitrates present in the leachings and water extracts from the soils in experiment 2. Possibly it was due to the utilization of nitrates by the nitrogen-assimilating organisms, or to the extremely small quantity of water-soluble phosphorus contained in this soil. McHargue and Peter [8] in their study of the removal of mineral plant food by natural drainage waters found that a high phosphorus content of soils accelerates the action of the nitrifying organisms.

EFFECT OF SULPHUR AND GYPSUM ON PHOSPHORUS

Analyses were made for phosphorus in the drainage water and leachings obtained from the soils in both experiments, but the amounts obtained were so small as to be well within the limit of error for this determination. These results are therefore omitted from the discussion. Most soils have shown the capacity to retain phosphorus compounds in the surface area, and usually only traces appear in the drainage.

TABLE VII.—*Amount of nitrogen in drainage water, leachings, and soil extracts*

Pot No.	Treatment (pounds per acre)	Experiment 1 (field pots)—nitrogen in pounds per 2,000,000 pounds of soil						Experiment 2 (greenhouse pots)—nitrogen in pounds per 2,000,000 pounds of soil		
		December	January and February	March and June	Total for five months	Soil extracts	Total water-soluble nitrogen	Leachings	Soil extracts	Total water-soluble nitrogen
1	Control	48.7	29.2	29.2	107.1	79.2	186.3	24.8	8.4	33.2
2	do	57.1	12.0	66.6	135.7	88.5	224.2	27.8	9.8	37.6
	Average	52.9	20.6	47.9	121.4	83.8	205.2	26.3	9.1	35.4
3	Uninoculated sulphur 186	58.3	18.6	14.6	91.5	55.9	147.4	21.1	8.1	29.2
4	do	54.5	15.0	15.0	84.5	51.2	135.7	23.7	7.3	31.0
	Average	56.4	16.8	14.8	88.0	53.5	141.5	22.4	7.7	30.1
5	Inoculated sulphur 189	59.3	15.9	16.0	91.2	60.5	151.7	17.3	7.0	24.3
6	do	51.1	15.1	16.2	82.4	55.9	138.3	21.6	5.8	27.4
	Average	55.2	15.5	16.1	86.8	58.2	145.0	19.4	6.4	25.8
7	Uninoculated sulphur 1000	58.6	17.8	11.6	88.0	51.2	139.2	18.0	7.8	25.8
8	do	56.0	13.6	18.6	88.2	55.9	144.1	24.2	8.4	32.6
	Average	57.3	15.7	15.1	88.1	53.5	141.6	21.1	8.1	29.2
9	Inoculated sulphur 1015	77.6	14.9	35.5	128.0	69.8	197.8	15.7	------	------
10	do	59.7	19.7	28.5	107.9	69.8	177.7	17.5	6.7	24.2
	Average	68.6	17.3	32.0	117.9	69.8	187.7	16.6	6.7	23.3
11	Gypsum 1000	52.2	15.3	12.7	80.2	55.9	136.1	16.8	5.0	21.8
12	do	57.1	16.1	13.3	86.5	55.9	142.4	24.3	6.7	31.0
	Average	54.6	15.7	13.0	83.3	55.9	139.2	20.5	5.8	26.4

[8] MCHARGUE, J. S., and PETER, A. M. THE REMOVAL OF MINERAL PLANT-FOOD BY NATURAL DRAINAGE WATERS. Ky. Agr. Exp. Sta. Bul. 237, p. 331-362, illus. 1921.

EFFECT OF SULPHUR AND GYPSUM ON HYDROGEN-ION CONCENTRATION

Table VIII gives the results of the determinations of the hydrogen-ion concentration in the samples of drainage water obtained from the pots in experiment 1 during the month of June, and in the leached soils at the close of the experiments.

Before the treatments were made the soil used in these experiments had a P_H of 7. The range of experimental error for the colorimetric method of determining hydrogen-ion concentration is usually considered to be 0.2 P_H. By taking this error into consideration and examining the determinations given for the duplicate treatments, it is noted that in every case at least one of these determinations falls within the 0.2 P_H limit. Therefore it can not be definitely said that any of the treatments increased the hydrogen-ion concentration in this soil or in the drainage water obtained during the month of June. It is surprising that considerable acidity did not develop in those soils receiving large additions of sulphur. A possible explanation for this fact is the high reserve supply of basic materials in the Palouse silt loam. As sulphuric acid was produced it was immediately neutralized by the bases present. Owing to its large supply of basic materials, this soil may receive considerable quantities of sulphur without showing the need of limestone to correct acid conditions.

The data given in Table VIII show also that there was insufficient acidity developed from sulphofication to bring about any solvent action on the native soil potassium and that the increase of this element, owing to the various treatments noted in Table IV, probably resulted indirectly from the formation of salts.

DISCUSSION OF THE CURVES

Each of the tables from III to VII, inclusive, gives a column representing the total water-soluble elements which were recovered in both experiments. The data giving the average of the duplicate treatments have been used

TABLE VIII.—*Hydrogen-ion concentration in the drainage water for June and in the soils at the end of the experiments*

Pot No.	Treatment (pounds per acre)	Experiment 1 (field pots)		Experiment 2 (green house pots)
		Drainage water for June	Soil extracts	Soil extracts
		P_H	P_H	P_H
1	Control	6.9	6.9	6.9
2	do	6.9	7.3	7.1
	Average	6.9	7.1	7.0
3	Uninoculated sulphur 186	6.7	7.2	6.7
4	do	6.7	7.3	6.5
	Average	6.7	7.1	6.6
5	Inoculated sulphur 189	6.6	7.4	6.7
6	do	6.7	7.0	6.9
	Average	6.65	7.2	6.8
7	Uninoculated sulphur 1000	6.7	7.1	6.7
8	do	4.6	6.9	6.6
	Average	5.65	7.0	6.65
9	Inoculated sulphur 1015	6.5	7.1	6.7
10	do	6.7	6.9	6.7
	Average	6.6	7.0	6.7
11	Gypsum 1000	6.7	7.4	7.3
12	do	6.8	7.2	6.9
	Average	6.75	7.3	7.1

in plotting the curves shown in Figure 1. The graph permits a comparison of the results obtained from both experiments, and also shows the relation of sulphofication to the availability of the soil potassium, magnesium, calcium, and nitrogen.

By examining the curves representing the different elements, and comparing the inoculated sulphur with the uninoculated sulphur, it is seen that, in general, the line connecting the two sulphur treatments is nearly always parallel to the abscissa. Especially is this true for the small applications of sulphur. It seems well established, therefore, that the uninoculated sulphur produced about the same effects in this soil as did the inoculated sulphur.

The curve representing the water-soluble calcium from the greenhouse pots follows the same general conformity as the sulphur curve for the greenhouse soils. This shows that with increased sulphofication there was a corresponding increase in the loss of calcium from the soils receiving sulphur, and this loss was greater with the high applications of sulphur than with the low sulphur treatments. The calcium curve for the field pots is apparently abnormal, or contrary to what would be expected to follow from the application of sulphur to the soil. The potassium curves show that increased sulphofication was followed by a greater availability of the soil potassium. Gypsum caused no marked effect on the water-soluble potassium, except under greenhouse conditions.

The curves representing the water-soluble magnesium obtained from the soils in both experiments show that the sulphur treatments had little effect on the loss of magnesium in the drainage water, except in the case of the large applications made on the greenhouse soils. The nitrogen curves likewise show that the applications of sulphur and gypsum had little effect upon nitrification.

CONCLUSIONS

From the data secured from this work on the Palouse silt loam, and presented in the foregoing tables and in Figure 1 (see p. 462), the following conclusions seem to be justified:

(1) The Palouse silt loam has a naturally high sulphofying power, and uninoculated sulphur when added to this soil was just as efficient as inoculated sulphur in producing sulphates.

(2) Under the conditions of the experiments the gypsum and sulphur used as fertilizers were readily leached out during the winter months. To guard against unnecessary losses of these materials it is advisable to apply them at a time when the crop has the greatest need for them.

(3) All of the sulphur treatments increased the availability of the native soil potassium, as is evidenced by the increased amount of this element in the drainage water from the soils which were treated with sulphur. Gypsum increased the amount of soluble potassium under greenhouse conditions, but definite increases in soluble potassium were not obtained under field conditions.

(4) Elemental sulphur, when oxidized in the soil under greenhouse conditions increased the loss of calcium in the leachings. The results on calcium obtained under field conditions were too irregular to point to any conclusions.

(5) Magnesium compounds in the soil were but little affected by sulphur or gypsum.

(6) Both the sulphur and gypsum treatments had little effect upon nitrification in this soil.

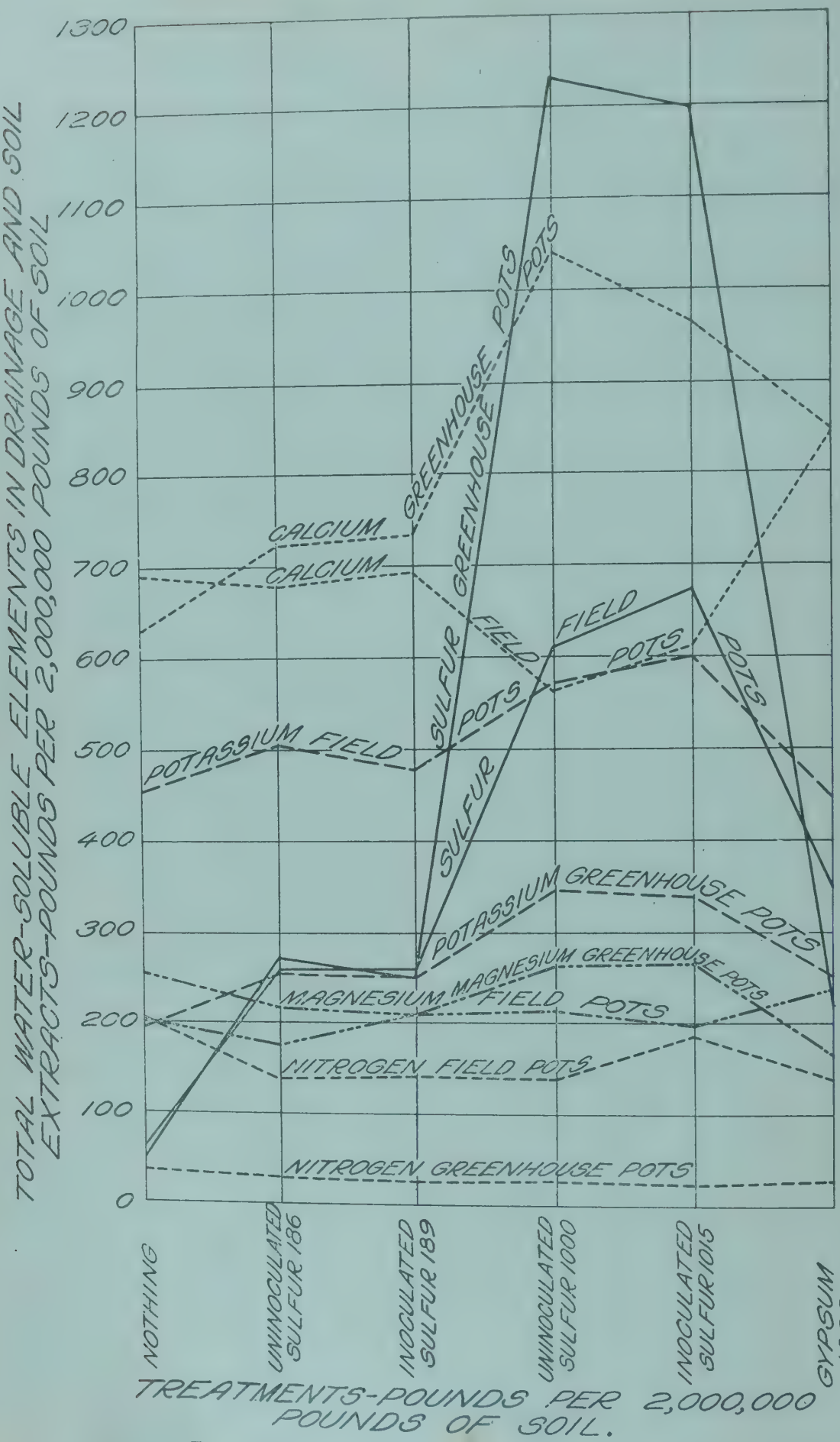

FIG. 1.—Effect of sulphur and gypsum on Palouse silt loam

PHYTOPHTHORA ROT OF PEARS AND APPLES[1]

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INTRODUCTION

On September 5, 1923, the junior author collected on the South Water Street Market, Chicago, Bartlett pears from Fennville, Mich., which showed black spots covering about half the pear. On the surface of these spots there was a scanty growth of mycelium (pl. 1, A). When the pears were cut through at these spots the flesh underneath was found to be for the most part very light brown in color but markedly darker brown in the vascular system (pl. 1, B). Mounts of the surface fungous growth under the microscope showed numerous conidia resembling those of Phytophthora, and plantings from the diseased flesh yielded a fungus which agreed in all important respects with published descriptions of *Phytophthora cactorum*. The pathogenicity of the fungus isolated from these pears and from apples and other pears collected later has been proved by inoculation into healthy pears and apples.

Further examination of fruits on the Chicago market showed that the disease is more widely distributed in the United States than had hitherto been suspected; a study of the fungus in culture and on the fruit brought out a number of facts concerning its morphology which seem not to have been observed by other workers. The present paper summarizes the results of this work and gives in addition a fuller description of the disease than is available in earlier publications.

DISTRIBUTION AND FIELD OBSERVATIONS

Following the collection of Phytophthora rot on the Chicago market on September 5, other collections were made as follows:

On September 11 a shipment of Clairgeau pears from St. Joseph, Mich., was seen on the Chicago market which showed a loss of about 30 per cent from a rot similar to that described above. It was learned later that the entire shipment consisted of windfalls.

On September 24 Anjou pears and on September 25 Clairgeau pears were found on the Chicago market which showed the symptoms of Phytophthora rot. Nothing was learned as to the origin of either of these lots of pears. It is probable, however, that they also came from Michigan, since they had been shipped in bushel baskets and Michigan was at that time the only Middle Western State shipping Anjou and Clairgeau pears in baskets into Chicago. Both varieties, if shipped from California or the Northwest, would have been packed in boxes.

On September 27 several orchards near St. Joseph, Mich., were visited and numerous cases of Phytophthora rot were found, on both pears and apples. The decay was found only on windfalls, never on fruit hanging on the tree. In some instances the windfalls under Clairgeau pear trees showed 100 per cent infection, particularly those under trees where there were indications that water had been standing on the soil. Moreover, pears which looked sound when viewed from above as they lay on the ground were usually found to have a black spot on the lower side if the ground was moist to the touch.

The varieties of pears found to be affected with Phytophthora rot were Clairgeau, Kieffer, Bosc, Howell, Bartlett, Anjou, and Angouleme (Duchess). The disease was also found to be serious among the windfalls on the following varieties of apples: Twenty-Ounce Pippin, Northern Spy, Tolman, Baldwin, Winesap, Jersey Sweet, Wealthy, Gideon, Rhode Island Greening, and Tompkins King. Cultures of a Phytophthora were obtained from all the varieties and all the collections mentioned above.

The influence of soil moisture was noted in two Kieffer pear orchards, both of which had been kept in clean culture. In one of these the soil was moist to the touch and practically all of the fruit on the ground showed the typical symptoms of Phytophthora rot. In the other orchard the surface soil

[1] Received for publication June 16, 1924; issued, May, 1925.

was almost dusty and only about 10 per cent of the windfalls showed the disease.

On October 29, 1923, the junior writer assisted in the inspection of a car of Delicious apples at Chicago, shipped from Wenatchee, Wash. Many of the apples were incrusted with clay, and one of them showed the characteristic symptoms of Phytophthora rot. A pure culture of Phytophthora was obtained from this apple. On January 11, 1924, the rot was found in Rome Beauty apples from Boise, Idaho, inspected in cold storage at Chicago. Plantings from affected fruits yielded only Phytophthora.

DESCRIPTION OF THE DISEASE

A brief description of Phytophthora rot on pears has been given in the first paragraph of this paper. However, there are certain characteristics of the rot on both pears and apples which merit further consideration, since a search of the literature shows them not to have been described in any detail before. Throughout the following discussion, which is based on a study of naturally infected fruits and of fruits inoculated in the laboratory, it is to be understood that all statements refer to tissues affected by the fungus. In both pears and apples there is always a marked vascular browning, both in the larger bundles near the core and in the smaller ones throughout the flesh (pl. 1, B and D). Very often the browning in the larger bundles extends to the stem and into it for part or all of its length (pl. 1, B) in the manner described by Schoevers (*12, p. 154*).[2] In this connection it may be mentioned also that Rose (*10*) found marked vascular browning in strawberries parasitized by a fungus very similar to and apparently identical with *Phytophthora cactorum*. In apples the flesh surrounding the bundles is lightly browned, in pears scarcely at all. In some varieties of pears, notably Clairgeau, the flesh is in fact decolorized and has a clear, water-soaked appearance very much like that of apple flesh affected by water-core. The parasitized flesh of both apples and pears sometimes becomes slightly spongy, but is usually as firm as sound healthy flesh; it rarely becomes soft and mushy like that found in lesions produced by Rhizopus or Penicillium. The only exception noted was that of pears of the Seckel variety, inoculated with Phytophthora in the laboratory. In cross-section, Phytophthora lesions on both pears and apples show indefinite boundaries so that it is impossible to make a clean separation of diseased from healthy flesh, as can so easily be done in fruits attacked by Rhizopus or Penicillium. The affected flesh has no marked odor or taste. Externally the lesions produced by Phytophthora on apples are light brown (pl. 1, C), on pears dark brown to black (pl. 1, A).

INOCULATION EXPERIMENTS

EXPERIMENT 1

Twelve Clairgeau pears were washed in soap and water and rinsed in sterilized tap water. They were then sterilized for 10 minutes in 1: 1,000 mercuric chloride solution, rinsed again in sterilized tap water, and placed in two moist chambers. Six were inoculated with a pure culture of Phytophthora obtained from Clairgeau pears and six were used as controls.

On the fourth day all the inoculated pears showed the characteristic lesions, and all the controls were free from any symptoms of the disease. The lesions ranged in size from 33 to 44 mm. in superficial diameter, averaging 37 mm. On the tenth day the controls were still in perfect condition, while the lesions on the inoculated fruits varied from 70 to 77 mm. in superficial diameter, averaging 74 mm. Cultures from these lesions yielded only Phytophthora.

EXPERIMENT 2

Eighteen apparently uninjured Kieffer pears were sterilized in 1: 1,000 mercuric chloride for 10 minutes. Six used for controls were placed in a moist chamber with water in the bottom.

A layer of cotton batting was placed in the bottom of a moist chamber and saturated with sterile water. On the cotton batting were placed six small pieces of an agar culture of the Phytophthora which had been isolated from Clairgeau pears. A sterilized pear was placed on each piece of agar culture. On the eighth day three of the pears, and on the thirteenth day all of them,

[2] Reference is made by numbers (italic) to "Literature cited," p. 468.

EXPLANATORY LEGEND FOR PLATE 1

A.—Anjou pear five days after inoculation with the Phytophthora isolated from a naturally infected pear
B.—Cross-section of the pear shown in A
C.—Grimes Golden apple five days after inoculation with the Phytophthora isolated from a naturally infected pear
D.—Cross-section of the apple shown in A (Color plate by J. Marion Shull)

C

A

B

D

showed the characteristic symptoms of Phytophthora. The fungus was recovered from two of these pears by culturing. All of the others were cut up and were found to show the characteristic vascular darkening.

EXPERIMENT 3

Some of the clay soil from an orchard at St. Joseph, Mich., was placed in a moist chamber and sterile water was poured over it until water stood on the soil to the depth of about 2 cm. Six sterilized pears, apparently free of skin punctures, were then placed in the moist chamber on the soil. All of these pears developed Phytophthora rot, and pure cultures of the organism were obtained from every one of them. The first pear developed the rot on the thirteenth day, two more on the twenty-first day, and the remaining three on the twenty-second day. Similar results were obtained in another experiment which duplicated No. 3, except that apples were used instead of pears. Controls provided in both experiments remained sterile.

Nothing is known of how infection occurred in the moist chambers containing pears resting on soil covered by water. Quite evidently the fungus existed in the soil and continued to grow there even though entirely submerged. It is possible and indeed quite probable that the fungus grew out into the water; but whether the infection which occurred was brought about by zoospores or directly by the mycelium is a question to which the experiments and the observations made on them give no answer.

The results obtained in these experiments are strong evidence that the Phytophthora isolated from apples and pears is able to penetrate the uninjured skin of those fruits. Evidence to the same effect was seen in the orchards visited in Michigan.

MORPHOLOGICAL FEATURES

Twenty of the isolations from diseased pears and apples, including the three from Washington and Idaho apples, were subjected to comparative study after they had grown for 9 to 70 days on potato dextrose agar, on Sherbakoff's oatmeal agar, and on oatmeal paste. On potato dextrose agar they all produce conidia (pl. 2, A and B) and oogonia (pl. 2, C to H) in abundance and also large numbers of bodies which are very similar to the "sphaero-conidia" (pl. 2, I to L) described and figured for *Phytophthora cactorum* by Lafferty and Pethybridge (*6, p. 37 and pl. II, fig. 13*). On oatmeal agar they produced large numbers of conidia and oogonia, but very few "sphaero-conidia"; in some cultures there were none of these bodies. Measurements of the various spores forms were made for three cultures, which so far as could be told by examination with the microscope were representative of the whole 20. One of these cultures had been isolated from the Delicious apple from Washington, one from a pear from Michigan, and one from an apple from Michigan. The measurements can be summarized as follows: Oospores, 15 to 43 μ in diameter, average 26.6 μ; conidia, length, 25 to 44 μ, average 34.4 μ; conidia, width 21 to 31 μ, average 26.8 μ; "sphaero-conidia," 25 to 45 μ in diameter, average 34.6 μ. All of these are in close agreement with the constants given for the spore forms of *Phytophthora cactorum* by other workers. (Rosenbaum (*11*) Beach (*1*) and measurements quoted by the latter from other papers.)

The conidiophores of this fungus have been found as (1) short branches of the mycelium bearing a single conidium, or (2) longer branches bearing conidia in groups of 2 to 10 or more (pl. 2, M). The largest number of conidia seen in a single group was 15. Both kinds of branches arise from the mycelium in the manner shown for one of the longer branches, in the lower portion of Plate 2, M.

All three of the spore forms mentioned above were found on the surface of both pears and apples collected on the market. They also appeared on the surface of pears and apples inoculated in the laboratory, usually within six or eight days from the time of inoculation. Favorable conditions for this external growth seemed to be a moist atmosphere and a temperature of 20° to 25° C. or even slightly higher. A few oogonia were seen in mounts of diseased tissue from the inside of a number of fruits.

Measurements of the various spore forms as found on diseased fruits can be summarized as follows: Oospores 20 to 35 μ in diameter, average 26.6 μ; conidia, length 30 to 53 μ, average 40.3 μ; conidia, width 19 to 32 μ, average 36.9 μ; sphaero-conidia, for the few that were seen, 26 to 38 μ in diameter average 32.3 μ.

It will be seen that the conidia are slightly longer on the host than on culture media but that the other two spore forms average about the same size no matter where they are produced.

For explanatory legend see p. 467.

Antheridia are usually paragynous (pl. 2, C and D) but in old cultures on oatmeal paste a few have been found which were amphigynous (pl. 2, E, F, G, and H). Lafferty and Pethybridge (*6, p. 36*) have already reported this condition for cultures of *Phytophthora cactorum* obtained by them from various sources, including one furnished by H. H. Whetzel and said by him to have been isolated from a decayed apple grown in his own garden in Ithaca, N. Y. Rose (*10*) has also reported it for a Phytophthora isolated from decaying strawberries and shown by him to be very similar to *Phytophthora cactorum*. The writers wish to call attention, however, to an unusual form with two amphigynous antheridia shown in Plate 2, H. As seen under the microscope, the antheridia lay in approximately the same plane. It was thought at first that there were two oogonia, one lying over the other; but after repeated and vigorous tapping of the cover glass the object still remained as figured on Plate 2, one oogonium with two amphigynous antheridia.

The oogonium is apparently intercalary, but even so it is difficult to understand how it could have grown through two antheridia in the manner described by Pethybridge (*9*) for growth through one. In other words, the finding of the form here under discussion (pl. 2, H) raises the question whether for the apple and pear Phytophthora antheridia in the amphigynous position have that position because the oogonium has grown through them. The writers have no other explanation of how antheridia may become amphigynous but will merely add that at no time during a rather extensive study of the apple and pear Phytophthora have they seen early stages of the growth of the oogonium through the antheridium. Antheridia in the amphigynous position have been seen only in the final, fully developed stage and only in old cultures or in the older portions of cultures that were a month to six weeks old.

It should be added that an oogonium with two amphigynous antheridia has been seen only once. Attention is called, however, to Plate 2, D and E, which shows two oogonia, each with one amphigynous and one paragynous antheridium. One of these aggregates lay in an open space between two masses of mycelium and was made to assume different positions by pressure on the cover glass. Study of it in these positions showed that the structures figured in Plate 2, D, were not merely in fortuitous juxtaposition but were actually parts of one whole. The other (pl. 2, E) lay in a matrix of agar and mycelium and was studied only in the position shown by the drawing. It would be possible of course for one antheridium to assume a position at the side of an oogonium after the latter had grown through another antheridium. The figures are included here merely on the chance that they may at some time help to explain the condition shown in Plate 2, H.

DISCUSSION

The finding of Phytophthora rot on fruits on the Chicago market is a new record, so far as the pathological work is concerned which is carried on in connection with the Food Products Inspection Service. That is, the rot has not heretofore been seen in the course of this work, or, if seen, has not been recognized. The finding of the rot on pears and apples from Michigan and on apples from Washington and Idaho is, so far as the writers are aware, a new record for each of those States, though not for the continent of North America. Whetzel and Rosenbaum (*15*) reported *Phytophthora cactorum* from New York on Duchess apples in 1916, Hesler (*14, p. 172*) from New York on apples in 1918, Güssow (*4*) from Canada on pears in 1919, Clinton (*3, p. 454*) from Connecticut on pears in 1919 and on apples in 1920 (*3, p. 406*), and Gardner (*5, p. 53*) from Indiana on apples in 1921. *Phytophthora cactorum* is a well-known parasite of pears and apples in Europe (Wormald, (*16*), Osterwalder (*8*), Lafferty and Pethybridge (*6*)

EXPLANATORY LEGEND FOR PLATE 2

Spore forms and sporophore of the Phytophthor isolated from apples and pears. (A, B, C, D, E, F, G, H, L × 785. J, K, I, M × 369)

A, B.—Conidia
C.—Oogonium with paragynous antheridium
D, E.—Oogonia, each with one paragynous and one amphigynous antheridium
F, G.—Oogonia, each with one amphigynous antheridium
H,—Oogonium with two amphigynous antheridia
I, J, K, L.—Various forms of Sphaero-conidia
M.—Conidiophore with conidia attached. Lower portion of the figure shows manner of origin from the mycelium

and other references cited below). The above account indicates that the rot of pears and apples caused by a Phytophthora is rather widely distributed in the United States. It is granted of course that the apples from Washington and Idaho are not known positively to have become infected with Phytophthora in the State where they originated. Nevertheless, in the absence of proof to the contrary, it is fair to assume that they did become infected there.

It is noteworthy that during this investigation Phytophthora rot was found in one lot, the storage lot of Rome Beauty apples from Idaho, approximately four months after the usual time for harvesting the variety named. Most of those who have studied this disease have found it either in fruit in the orchard or in fruit which had been taken from the orchard only a few days before. (Bubak (*2*), Clinton (*3*), Güssow (*4*), Hesler (*14*), Marchal (*7*), Schoevers (*12*), Unamuno (*13*), Whetzel and Rosenbaum (*15*), and Wormald (*16*).) The apples in which Lafferty and Pethybridge (*6*) found the rot had been picked on October 14 and were sent to them on November 20. The authors make the statement (*6, p. 29*) that it is not known whether infection occurred prior to gathering or in storage. Osterwalder (*8, p. 440*) reports that he did not see the fungus at any time on stored apples. From the context the natural inference is that he is referring to the rot.

What was thought to be *Phytophthora cactorum* was also reported in 1922 from Pennsylvania on growing apple fruits by Thurston (*14*).

SUMMARY

There has been found on the Chicago market in pears and apples from Michigan and in apples from Washington and Idaho a rot from which a species of Phytophthora has been isolated.

This Phytophthora when inoculated into healthy pears and apples reproduced the diseases and was easily recovered from the affected tissues.

Evidence is presented which indicates that the Phytophthora isolated from pears and apples is similar to and probably identical with *P. cactorum*.

The observations of Lafferty and Pethybridge (*6*) are confirmed, that the apple and pear Phytophthora produces "sphaero-conidia" and both paragynous and amphigynous antheridia.

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SOME PHYSICAL AND CHEMICAL PROPERTIES OF CAROTIN AND THE PREPARATION OF THE PURE PIGMENT [1]

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INTRODUCTION

In a previous paper (*6*)[2] certain colorimetric and spectrophotometric data have been given for carotin. By using the graphs from these data the quantitative results presented herein have been obtained. The method of procedure for the preparation of the pure carotin used in obtaining the data for the graphs and the solubility determinations is given in detail in this paper.

In connection with the solubility and with the quantitative determinations, it is essential to know what effect the solvent has upon the stability of the pigment in solution. Consequently, the keeping qualities of solutions of carotin have been studied.

Some idea of the practical application of the data contained in this paper and in the previous one may be obtained by a brief examination of Palmer's book on "Carotinoids and Related Pigments." The data presented should be of especial value to those engaged in the manufacture and purification of vegetable oils, for the pigments present in the vegetable oils (*5*) are closely related in spectral transmissive properties to the carotinoids, and very likely they consist of carotin and xanthophyll. Parsons and Wilson (*4*) have shown in Plate 1, page 270, of their paper [3] the absorption curves for petroleum oils. These curves show very clearly that it is possible to grade the oils when the transmissivity of the oil in question is known.

SOLUBILITY OF CAROTIN

Absolute ether distilled over sodium, ethyl alcohol (99.7 to 100 per cent), and redistilled petroleum ether (benzin petroleum) which fractionates at 30° to 50° C., were the solvents used in the solubility tests. Impurities in the ether (*1*) affected the solubility of carotin so that it was necessary to allow the ether to stand over sodium for several days and then freshly distill just enough for use.[4]

All of the solubility tests reported were made in a constant-temperature water bath which was kept at 25° C. The time the solutions remained in the bath and the amount of stirring varied somewhat, but it is stated for each set of determinations.

Either crystals of carotin freshly prepared, as will be described later in this paper, or those which had been kept in absolute alcohol in a sealed vial after filtering off and washing with low-boiling petroleum ether were used. They were dissolved in carbon disulphide or chloroform, and low-boiling petroleum ether was added to this solution. Crystals were obtained from this solution by evaporating the solvents under reduced pressure. The crystals were collected on a hardened filter in a small Büchner funnel and quickly washed two or three times with small portions of low-boiling petroleum ether, after which they were dried for 15 to 30 minutes in a well-evacuated desiccator. The melting point was quickly taken and if found satisfactory (174°) a small portion of the crystals was placed in each of two carefully cleaned bottles, which were partially filled with their respective solvents—alcohol or petroleum ether—and placed in the water bath for three hours, during which time the bottles were frequently shaken.

In most cases 10 c. c. of the saturated solution were withdrawn from the bottle which had been kept in the water bath. By carefully tying a filter paper on the end of a pipette, 10 c. c. of the saturated solution of carotin could be

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[1] Received for publication June 6, 1924; issued May, 1925. This paper is a report of an investigation on the four chloroplast pigments.

[2] Reference is made by number (italic) to "Literature cited," p. 474.

[3] Spectrophotometric methods would undoubtedly be far more accurate than the method which they describe, and in addition would be simpler. The fact that all of the paraffin base oils (light and dark) have essentially the same light absorption curve makes the spectrophotometric methods even more useful in determining the grade of the oil.

[4] The impurities probably were peroxides, which may be removed by distilling the ether over sodium.

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withdrawn without removing any of the crystals. The saturated alcoholic solution as a rule was made up to a volume of 50 c. c., or diluted 5 times; the saturated petroleum ether solution was diluted 100 or 200 times, and the saturated ether solution was diluted 1,000 times for the spectrophotometric determinations. In every case U. S. P. ether was used in making the dilutions, which were all made at room temperature a few minutes before the spectrophotometric readings were taken in a 2 cm. cell. The scale on the spectrophotometer was set so as to use the mercury line 435.8 mμ, in which position readings were made.

The quantity of pigment in the various solutions was determined at room temperature by means of a König-Martens spectrophotometer in the manner described in a previous paper (*6*).

Experiments 3, 4, 5, 6, 7, and 8 of Table I were made in a slightly different way than 1 and 2. In these cases the solubility tests were made in 30 c. c. bottles placed inside a fruit jar which was closed tightly so that no water might enter. The jar and its contents were then mechanically revolved in the water bath for the time specified.

In experiments 6, 7, and 8 the carotin crystals used were taken from the same preparation of carotin, the other experiments being on different samples.

TABLE I.—*Solubility of carotin at 25° C. in absolute alcohol*

Experiment No.	Dilution	Approximate time in water bath	Transmittancy	Carotin per liter
	Times	*Hours*		*Mgm.*
1	5	3	0.2170	8.3
2	5	12	.1700	9.6
3	5	15	.1920	9.0
4	5	20	.0673	14.8
5	5	20	.0572	15.6
6	5	48	.0718	14.3
7	5	72	.0371	18.0
8	5	90	.0693	14.6

It appears that after 20 hours saturation was reached in the alcohol solution; and, by averaging all of the figures for the solubility at 20 to 90 hours in the water bath, the solubility for carotin in absolute alcohol is 15.5 mgm. per liter at 25° C.

By averaging the results for 48, 72, and 90 hours in Table II, it is found that about 626 mgm. of carotin dissolved in one liter of petroleum ether at 25° C.

TABLE II.—*Solubility of carotin at 25° C. in petroleum ether, B. P. 30° to 50°*

Experiment No.	Dilution	Approximate time in water bath	Transmittancy	Carotin per liter
	Times	*Hours*		*Mgm.*
1	100	3	0.0610	305
2	100	15	.0310	382
3	100	20	.0200	428
4	200	20	.0890	526
5	200	48	.0634	604
6	200	72	.0724	570
7	200	90	.0400	704

The solubility in ether was obtained with difficulty, on account of the instability of carotin in ether solutions, as already mentioned.

Table III shows the solubility of carotin in specially purified ether. At 30 hours the equilibrium seemingly has become established and the average results show that approximately 1,005 mgm. of carotin are soluble in a liter of ether at 25° C.

TABLE III.—*Solubility of carotin at 25° C. in absolute ether*

Sample No.	Dilution	Approximate time in water bath	Transmittancy	Carotin per liter
	Times	*Hours*		*Mgm.*
1	1,000	30	0.402	980
2	1,000	46	.377	1,050
3	1,000	54	.398	1,010
4	1,000	90	.402	980

Boiling ether was found by Willstätter (*8*) to dissolve about 1.11 gm. of carotin per liter and low-boiling petroleum ether on a reflux condenser to dissolve 0.66 gm. per liter, while he observed that carotin was very difficultly soluble in absolute alcohol.

STABILITY OF CAROTIN IN SOLUTION

A small quantity of pure carotin was placed in each of three flasks; absolute alcohol was added to one, to another absolute ether (not freshly distilled), and to the third petroleum ether (30° to 50°). The solvents used were of the same grade as those employed in the solubility tests above, except that the ether was taken directly from the container in which it came.

These solutions were stored in the ice box and from time to time the transmittancy was determined spectrophotometrically. Dilution was often found necessary, because the instrument is best adapted for use with carotin solutions of low concentration.

In Table IV are presented results obtained with solutions of carotin in alcohol, in ether, and in petroleum ether, when kept for varying lengths of time.

TABLE IV.—*Spectrophotometric determinations showing the stability of carotin in alcohol, in ether, and in petroleum ether when stored in the ice box for varying lengths of time*

Number of days	Transmittancy	Solution	Dilution	Carotin per liter
			Times	*Mgm.*
0	0.0403	Absolute alcohol	×1	3.64
	.1360	Absolute ether	×20	43.60
	.0976	Petroleum ether	×20	50.80
24	.0424	Absolute alcohol	×1	3.58
	.0991	Absolute ether	×4	10.12
	.0149	Petroleum ether	×10	46.00
38	.0386	Absolute alcohol	×1	3.68
	.0557	Absolute ether	×2	6.32
	.1050	Petroleum ether	×20	49.00
50	.0408	Absolute alcohol	×1	3.63
	.0513	Absolute ether	×1	3.24
	.1047	Petroleum ether	×20	49.00
71	.0420	Absolute alcohol	×1	3.60
	.1510	Absolute ether	×1	2.05
	.0982	Petroleum ether	×20	50.40
111	.0439	Absolute alcohol	×1	3.55
	.3420	Absolute ether	×1	1.16
	.1020	Petroleum ether	×20	50.00
143	.0444	Absolute alcohol	×1	3.54
	.4610	Absolute ether	×1	.83
	.0906	Petroleum ether	×20	52.40

The results obtained in Table IV are graphically shown in Figure 1. Carotin apparently is just as stable in petroleum-ether solution as it is in alcohol solution. The rapid decline in the ether curve at once indicates that decomposition of the carotin in the ethereal solution has been proceeding during the course of the experiment. The oxidation of the carotin may be due to the formation of peroxides (*1*) in the ether, as already pointed out.

These facts are of importance to anyone who is interested in keeping carotin solutions for any purpose whatsoever. The data for the alcohol solution show that it is possible to make up a standard solution of pure carotin in absolute alcohol and keep it for months, during which time it may be used for comparative work. The facts as observed are also of importance in connection with the method of determining the amount of carotin in any substance—for example, in green leaves. In the method of separating the plant pigments, carotin is obtained in solution in petroleum ether. This solution of carotin in petroleum ether may be stored in the ice chest for several days without fear of decomposition before making spectrophotometric, colorimetric, or comparison determinations of the amount of carotin which it contains. This is a decided convenience if a great number of determinations are to be made with a colorimeter, for such determinations can not be made at all on some days, nor at any time during the day, because of light conditions.

SEPARATION AND PURIFICATION OF CAROTIN FROM CARROTS

Carotin may be obtained from carrots in the manner described below:

After careful washing to remove any adhering dirt or foreign material, the carrots are sliced in a power slicer or by hand, using a vegetable slicer; the knives should be sharp, so as to injure the tissue as little as possible. The cut pieces should be about 4 mm. thick. The sliced carrots are placed on a large galvanized-iron wire tray and dried in an oven in which the temperature is never allowed to exceed 50° C.

Since destruction of a large percentage of the carotin takes place during drying, the process should be carried on as rapidly as possible till the carrots are dry enough to grind in a power feed mill. The finely ground carrots will probably need to be dried further for several hours in a vacuum oven at less than 50° C. Grinding is rendered difficult if the material is not fully dry, as the presence of sugar in the carrots tends to clog the mill. After the final drying in the vacuum oven, the carrot meal is further ground in a ball mill (about 12 hours) till the greater part will pass a 40-mesh sieve. The finer it is ground, the more slowly the solvent will percolate and the higher will be the yield of the extracted pigment.

The pulverized carrots (3 kgm.) are placed in a glass percolator (10 cm. in diameter at the top and 60 cm. long) provided with a layer of cotton in the bottom to prevent the fine red powder from going through, and the percolator is then attached to a large suction filter flask. Pure redistilled petroleum ether (B. P. 30° to 60° C.) is added and suction is applied till the solvent just begins to run through. More petroleum ether is then added and allowed to percolate slowly overnight without using suction, when a large quantity of the colored extract will have passed

through into the flask. More petroleum ether is added and reduced pressure is applied till all of the colored extract has been removed. About 7 liters of petroleum ether are necessary to extract the 3 kgm. of dry powder which represent a little over 1 bushel of fresh carrots. From 1 bushel (50 pounds) of carrots, 2,774 gm. of dry carrot powder ready for extraction are obtained. In all, an afternoon and the following morning are required to complete this extraction.

The total red-brown percolate, from which crystals may be already separating, is now concentrated on a steam-heated water bath, the temperature of which is never allowed to exceed 50° C. About half of the petroleum ether used may be recovered by careful distillation. The concentrated petroleum ether solution is further concentrated under reduced pressure till most of the carotin has separated in the crytalline form. During the last stages of concentrating a fine current of carbon dioxide which has been led through a calcium chloride drying tube and then into the flask through a capillary is advantageous, because it prevents frothing over, aids evaporation, and also keeps the solution under an inert gas. In addition, glass beads are added to prevent bumping. After

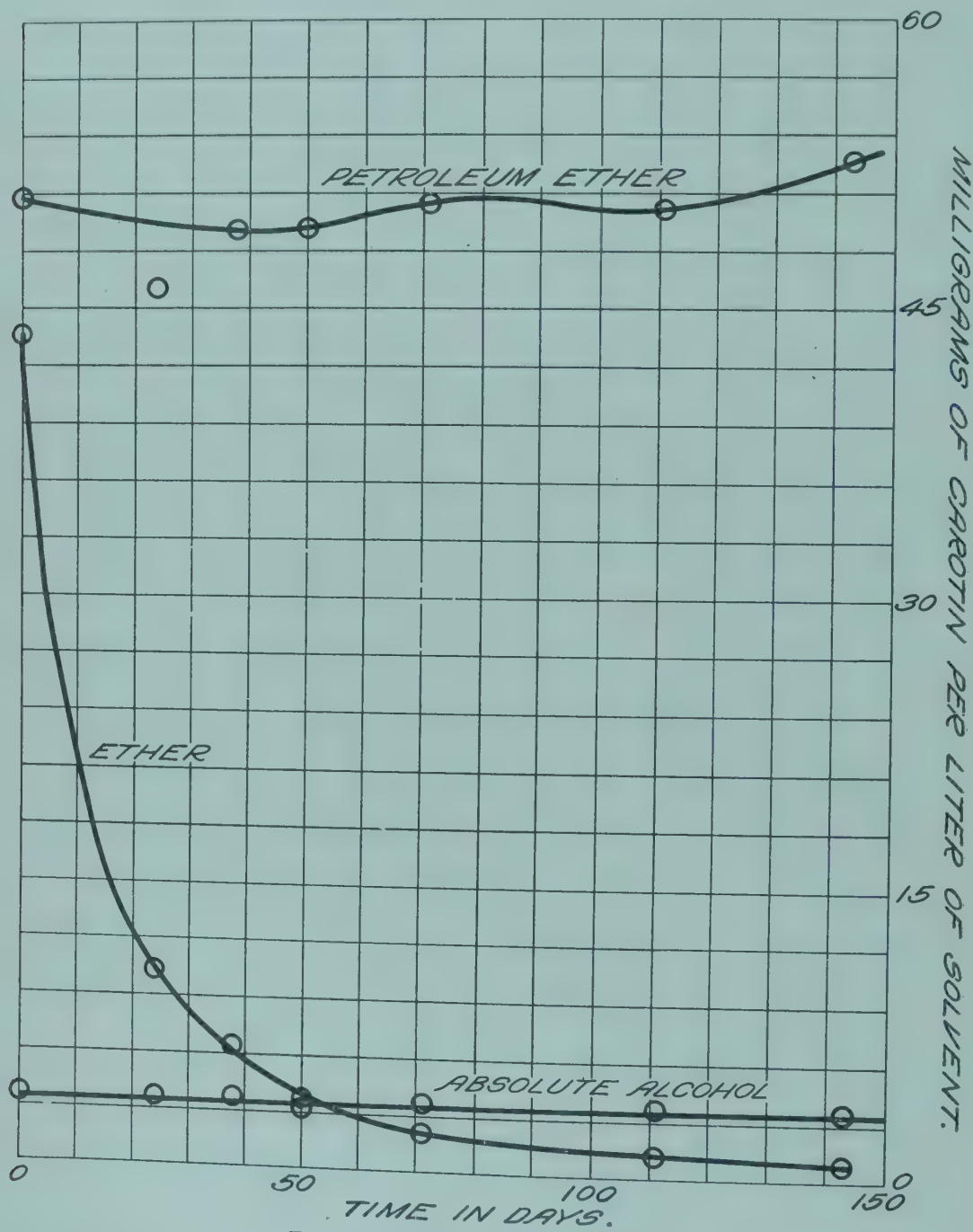

FIG. 1.—Stability of carotin in solution

concentrating to about 250 c. c., a stronger suction is applied so as to evaporate the petroleum ether and cool the solution in order that more of the carotin will crystallize; during this evaporation the flask is removed from the water bath to cool the solution. Or, after concentrating to 250 c. c., the concentrated carotin solution may be placed in an ice chest. In two or three days most of the carotin will have crystallized from the solution. The crystals are then filtered off.

After separating the gold, shimmering mass from the mother liquor by filtration, the crystals are dissolved on the same filter in the least possible quantity of chloroform or carbon disulphide, from which they are precipitated by shaking vigorously with small portions (50 to 100 c. c. in all) of absolute alcohol. Greatly reduced pressure may be used here, which will cause the chloroform or carbon disulphide to evaporate and cool the solution. Both the cooling and the evaporation of the solvent aid in crystal formation and cause a larger yield. After about one hour the crystals are filtered off either on a filter paper or a linen cloth; the flask in which the precipitation took place is rinsed with low-boiling petroleum ether (25 to 50 c. c.), and this is used to wash the crystals which are on the filter paper or cloth in the Büchner funnel. This washing will dissolve most of the remaining fats and waxes. The crystals are washed again with low-boiling petroleum ether and suction is applied till they are free of this solvent. Willstätter washes here with a half-and-half mixture of alcohol and petroleum ether; this mixture has the advantage that it does not dissolve as much of the crystals and the disadvantage that it does not dissolve the impurities as well as pure petroleum ether.

To avoid the possibility of the carotin crystals containing alcohol of crystallization (*7*) they are recrystallized by dissolving in just enough chloroform or carbon disulphide to effect solution and then adding petroleum ether (30° to 60°) and evaporating to about 25 c. c. or less. The crystals are collected on a hardened filter paper and washed two or three times with 10 to 15 c. c. portions of low-boiling petroleum ether. Any traces of fats, waxes, or oils should be removed by this last precipitation and final washing with petroleum ether. The pure pigment thus obtained is dried for 15 to 30 minutes in a good vacuum desiccator. The melting point is determined and, if found satisfactory (174°), the samples desired are rapidly weighed, placed in glass tubes filled with CO_2, sealed or dissolved for use, and the remaining crystals stored immediately, as described below.

Carotin crystals in mass are colored dark copper-red. When viewed through the microscope they have an orange-red color (*3*). The color and crystal form are illustrated by Escher (*2*) and the crystal form by Willstätter and Stoll (*8*). Carotin nearly always crystallizes in plates or leaves which often are rhombic or quadratic, and rarely does it crystallize in needles. The form and the size of the crystals vary with the solvent, the amount of the impurities in the solution, the temperature, and the time allowed for crystallization.

Very dilute solutions of carotin in alcohol, ether, or petroleum ether are yellow, more concentrated ones are deep orange, and very concentrated ones are deep red, except in the case of alcohol where the saturated solution is yellow. A weak solution of carbon disulphide is pink to red, whereas the more concentrated ones are red, dark red to almost black.

Escher (*2*) has obtained 125 gm. from 5,000 kgm. or 1 gm., of carotin from 40 kgm., (equivalent to 0.56 gms. per bushel) of fresh carrots, which is perhaps the best yield reported in the literature. On a small scale, Escher obtained from carrots which he himself dried, carotin at the rate of 0.10 gm. per kgm. of fresh carrots (equivalent to 2.27 gm. per bushel). He determined colorimetrically that carrots contained as much as 0.023 per cent of their fresh weight as carotin, or approximately 5.22 gm. per bushel, whereas the same variety when dry contained 0.013 per cent (based on fresh weight) of carotin or 2.95 gm. per bushel. Hence, there was a loss of 43.5 per cent of the carotin during the drying process.

In this investigation, 1 bushel (50 pounds) of fresh carrots contains from 1.68 to 3.46 gm. of coloring matter estimated colorimetrically as carotin. The same carrots when dried and ground in a ball mill ready for extraction contained respectively 1.41 to 2.81 gm. of carotin (based on a bushel of fresh carrots). From the bushel of carrots which contained 2.81 gm. of carotin when dry, 1.13 gm. of pure carotin, crystallized from petroleum ether and having a melting point of 174° C., were obtained. The loss in drying, 16.1 and 18.8 per cent, respectively, is probably explained by the manner of slicing the carrots.

Some fresh carrots were sliced and some were ground. Carotin decomposition was much greater in the ground carrots owing perhaps to the greater mechanical injury to the tissues in the grinding process, greater exposure of the material to oxygen, and slower drying. Incomplete extraction and losses in the crystallizations are further responsible for the low yield obtained.

SUMMARY

(1) The solubility of carotin at 25° C. in absolute alcohol was found to be 15.5 mgm. per liter; in petroleum ether (B. P. 30 to 50° C.), 626 mgm. per liter; in specially purified ethyl ether, 1,005 mgm. per liter.

(2) Solutions of carotin in absolute alcohol and petroleum ether were found to be extremely stable when kept in the ice box. An ether solution of carotin decomposed rapidly though stored in the ice box. The decomposition may have been caused by peroxides in the ether.

(3) Crystals of carotin may be stored in alcohol or petroleum ether for some time with only slight oxidation even though the container is not sealed, and may be stored permanently in these solvents if sealed in ampules.

(4) The method for the preparation of pure carotin from carrots on a small scale is described.

(5) A bushel of carrots (50 pounds) which contained 3.46 gm. of carotin colorimetrically when fresh, and 2.81 gm. of carotin colorimetrically when pulverized for extraction, yielded 1.13 gm. of pure carotin (M. P. 174°).

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THE LIFE HISTORY OF THE TEXAS ROOT ROT FUNGUS, OZONIUM OMNIVORUM SHEAR [1]

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The fungus now generally accepted as the cause of root rot of cotton and a large number of other cultivated and wild plants in Texas and other parts of the Southwest as far as southern California was first described by Pammel [2] and tentatively referred to *Ozonium auricomum* Link. This fungus seems to be a native of the southwestern United States and is most common in the so-called "black waxy soils" of Texas; but it seems to be becoming more widespread and more serious in southwestern Arizona in recent years with the increased growing of alfalfa and cotton there.

Investigations of this fungus made by the writer in the period 1902 to 1907 and the comparison of it with Link's type of *O. auricomum* in Berlin indicated that the organism was quite different from Link's species, and as no description which seemed to apply to it could be found in the literature it was described and named *Ozonium omnivorum* Shear.[3]

At that time the fungus was known only as a sterile mycelium. Circumstantial evidence, however, based upon the occurrence of a conidial fungus producing spores in great abundance on the surface of the soil in portions of cotton fields where the plants had been killed by the fungus, suggested that this might be a sporogenous form of the Ozonium.

Duggar,[4] in 1916, reported the presence of conidia-bearing hyphae on the characteristic mycelium of the Ozonium found on the roots of affected plants, and also the identity in artificial culture of the mycelium obtained from diseased roots with that obtained from cultures of these conidia.

The conidial condition of the fungus (fig. 1) studied by Duggar was referred to the genus Phymatotrichum of the Hyphomycetes and the new combination *Phymatotrichum omnivorum* (Shear) Duggar proposed.

Taubenhaus and Killough [5] state that the senior author has confirmed Duggar's work by producing conidia of the Ozonium in pure culture on sterilized soil in the laboratory. Only one out of six cultures, however, produced conidia. As this spore form belongs to the so-called imperfect fungi which are believed to be merely conidial conditions of the higher forms, it was natural to suppose that a perfect stage of the fungus might still occur, though perhaps rarely.

In hope of determining the complete life history of the fungus, numerous cultures were made on different media and kept under various conditions for long periods, but they always remained sterile. Careful observation and search in diseased areas on cotton roots and stems which had been killed by the fungus were made at different seasons, especially in the winter, spring, and early summer. Numerous fungi were, of course, found but none could be proved to belong to the root-rot fungus. In August, 1903, however, the writer found what he believes to be the perfect stage of this Ozonium, although the evidence at present is circumstantial. In a cotton field near Paris, Tex., there was a so-called "dead spot" of cotton in which the plants had all been killed by this fungus, and immediately adjoining this spot was a hedge of osage orange (*Maclura aurantiaca* Nutt.). In the margin of this diseased area, not far from the dead and dying cotton plants, was a small sprout of the osage orange about 1 foot high which showed the characteristic wilt produced by the Ozonium. An

[1] Received for publication June 27, 1924; issued May, 1925.

[2] Pammel, L. H. COTTON ROOT-ROT. Tex. Agr. Exp. Sta. Ann. Rpt. (1889) 2 : 73, pl. 1–3. 1890.

[3] Shear, C. L. NEW SPECIES OF FUNGI. Bul. Torrey Bot. Club 34 : 305. 1907.

[4] Duggar, B. M. THE TEXAS ROOT ROT FUNGUS AND ITS CONIDIAL STAGE. Ann. Mo. Bot. Gard. 3 : 11–23 illus. 1916.

[5] Taubenhaus, J. J., and Killough, D. T. TEXAS ROOT ROT OF COTTON AND METHODS OF ITS CONTROL, Tex. Agr. Exp. Sta. Bul. 307, p. 5, illus. 1923.

examination of the wilting plant showed that the typical mycelial strands of yellowish hyphae of Ozonium extended from the soil up the stem several inches and there began to change into a subiculum which surrounded the stem and formed a hymenium with the typical spines of a species of Hydnum, as illustrated in the accompanying plate (pl. 1). The organic connection between the typical Ozonium hyphae and those of the Hydnum appeared to be unquestionable.

In order to complete the proof of the genetic relationship of the two forms, efforts were made to grow the fungus from the Hydnum. Unfortunately, however, all of our efforts in this direction failed.

mycelium has a general similarity to the various sterile mycelia described by Persoon under the generic name Fibrillaria, some of which are known to belong to hymenomycetous fungi.

Being unable to find named specimens or a description to apply to this Hydnum, a description and illustration are given which it is hoped will make it possible for mycologists and pathologists who may encounter the fungus to recognize it.

Hydnum omnivorum n. sp.

Subiculum effuse, thin, readily separating from the substratum, subgelatinous, pale yellow, margin floccose, fibrillose-fimbriate, white; spines slender, acute, somewhat crowded, 2–3 mm. long, orange-buff to light orange-yellow (Ridgway). No spores found.

Conidial stage (*Phymatotrichum omnivorum*). Hyphae forming a loose layer on the surface of the soil in areas where the root rot has killed the plants; fertile hyphae arising irregularly from the mycelium, simple or forked; conidia numerous, sessile, borne on the irregularly swollen clavate or subglobose

FIG. 1.—*Phymatotrichum omnivorum:* Conidial stage of the fungus, showing mode of conidial production (after Duggar)

Conidial forms similar to the Phymatotrichum stage of this fungus are known to occur among the Hymenomycetes and there is nothing in this life history which conflicts with our present knowledge of the subject.

The Hymenomycete, *Tomentella granulata*, according to Brefeld [6] has a conidal form very similar to that of *Ozonium omnivorum*. The Ozonium

[6] BREFELD, O. BASIDIOMYCETEN III. p. 11–12, tab. 1, fig. 16. 1889. (*In his* Untersuchungen aus dem Gesammtgebiete der Mykologie, Heft 8.)

EXPLANATORY LEGEND FOR PLATE 1

Life history of Ozonium omnivorum Shear
A.—Hyndum omnivorum. Natural size on wilting Maclura
B.—Portion of the fungus, with spines enlarged 20 diam.

A
B

short terminal or lateral branches of the hyphae or on the sides of the undifferentiated hyphae, pale ochraceous to gray in mass, appearing nearly colorless when separate, globose to ovoid 4 to 6×5 to 8 μ, smooth.

Sterile mycelial stage (*Ozonium omnivorum*) mycelium thin, floccose to arachnoid, forming thin branching strands, the dirty ochraceous branches arising from these strands are thin, somewhat rigid and branch at right-angles from somewhat swollen nodes, 2 to 4 branches from a node, usually long, thin, tapering and acute (fig. 2).

Type No. 5267 C. L. S. on wilting *Maclura aurantiaca* near Paris, Tex., September, 1903. In herbarium pathological collections, Bureau of Plant Industry, U. S. Department of Agriculture.

This plant has something of the general appearance of some of the yellow resupinate species of Hydnum which have been described, but appears to be distinct, especially in its parasitic habit and apparent restriction to the southwestern United States and Mexico, the only region where this root rot is known. The perfect stage probably occurs as a saprophyte also, as specimens very similar, if not identical, have been found on old over-wintered cotton stalks killed by the root rot.

Unfortunately the character of the basidiospores can not be given, as none could be found on the specimen described. The enlarged spines in the plate had the tips broken off in most instances. When whole they are acute, as indicated in a few cases. The color in fresh specimens is brighter than in the dried, as it fades in drying.

FIG. 2.—*Hydnum omnivorum:* Ozonium stage, showing characteristic modes of branching of the terminal hyphae. × 420

THE DISTRIBUTION OF THE ALFALFA WEEVIL (PHYTONOMUS POSTICUS GYLL. A STUDY IN PHYSICAL ECOLOGY[1]

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INTRODUCTION

The alfalfa weevil (*Phytonomus posticus* Gyllander) is a recently introduced insect which is doing a large amount of damage in the Great Basin region. It was first noted near Salt Lake City, Utah, in 1904, and has spread until up to the present time it has been captured in Oregon, Idaho, Wyoming, Colorado, Nevada, and California, as well as in Utah. In many parts of its present range it is capable of doing an immense amount of damage, at times almost completely destroying the alfalfa crop. Although the area now occupied by the insect is a small portion of the total area devoted to alfalfa growing in the United States, the insect is regarded with alarm by all of the neighboring States, which have enacted quarantine laws against alfalfa in an effort to retard the further dispersion of the weevil.

At the present time the insect is rapidly spreading into new territory, and it would be a matter of great interest and value to determine, with some degree of accuracy, the probably final limits of its distribution in America. This paper is an attempt to determine, from the climatic limitations found in Europe and Asia, the probable economic distribution of the alfalfa weevil in America. The study will be presented in three parts: (1) A compilation of all known information concerning the effects of climatic factors on the life history and abundance of this species in America; (2) a study of its distribution in Eurasia, to determine the optimum and limiting climatic conditions; and, (3) the application of this information to a study of climatic conditions in America, to locate the regions of optimum and limiting conditions, and to outline the distribution.

CLIMATIC ECOLOGY OF PHYTONOMUS POSTICUS IN AMERICA

The data which are quoted in this portion of the paper have been taken from various papers on the alfalfa weevil, and the material has been quoted in full in each case, following the authority, which is placed in parentheses.

Outline of Life History

The alfalfa weevil winters in Utah as an adult in trash and weeds in fields. Early in the spring the adults emerge from hibernation and fly about actively, seeking a place for oviposition. This is known as the spring flight and occurs in April and early May. Oviposition continues into June, reaching a maximum late in May. The majority of the larvae are found feeding on the alfalfa leaves in early June in Utah. They pupate in June, and the adults emerge in early July to feed and fly around for a month or so (summer flight), after which they appear to become much less active, finally hibernating in the fall. There is only one complete generation per annum. In Utah there is sometimes a partial second generation, but the larvae do not survive the winter. The larvae strip the leaves from the first crop of alfalfa, and after that is cut they keep down the sprouts of the second crop until late in June, so that this crop is greatly reduced, and the third crop is so late as to be of little value.

Direct Relations of Climatic Factors to the Life History

TEMPERATURE

Reeves et al. (*9, p. 91*)[2] state: "There is no definite hibernation in this species. The adults are quiet when it is cold and active when it is warm . . ."

Titus (*14, p. 108*), says: "The time of entering hibernation varies greatly in different years and in different localities. If the summer is long, with warm, sunshiny weather throughout the latter part of the season, the weevils enter hibernation late and many of them perish before the summer ends. On the other hand, if the late

[1] Received for publication June 11, 1924; issued May, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 492.

summer is rather cool and cloudy, they will go into hibernation earlier, and apparently more of them pass through the winter successfully. In the higher mountain valleys the weevils become adult later in the summer and enter into hibernation earlier."

Apparently the rate of oviposition is closely related to temperature. Parks (*7*) has published detailed information upon the relation of temperature to oviposition, and it is evident from his chart (*7, p. 420*) that there is very little oviposition until the mean daily temperature reaches about 50° F. Titus (*14, p. 113*) makes the following statement, which is substantiated by Parks:

"The length of the egg-laying period each year naturally has a decided effect on the length of time that appreciable injury will occur to the crop for that year. Warm, dry, spring weather appears to be conducive to rapid egg laying, and thus many more larvae are feeding on the alfalfa at any one time than when the egg-laying period is extended over several months. Under these conditions more larvae come to maturity and the damage to the crop is more severe, since so many worms are present that there is little chance for the plant to recuperate. On the other hand, a slow cold spring and summer means that egg laying will continue for many weeks, and though probably as many larvae actually feed throughout the season, the damage to the crop harvested is not so great, the plant continually having opportunity to develop new buds and leaves. This readily explains the apparent decrease in injury in infested regions in 1912 as compared with the loss in 1911 in the same sections of the State."

The following table shows conditions in the spring months at Salt Lake City in 1911 and 1912:

Month	Mean temperature		Total precipitation	
	1911	1912	1911	1912
	°F.	°F.	*Inches*	*Inches*
March	44.4	40.0	2.04	3.48
April	47.9	46.8	1.65	2.34
May	57.2	55.8	1.84	1.75
Means and totals	49.8	47.5	5.53	7.57

These figures, which substantiate the above statement so far as climatic conditions are concerned, are taken from the Annual Meteorological Summary (*16*) for Salt Lake City for 1921.

The following notes regarding the influence of altitude upon damage are of particular interest when considering temperature.

Ball (*1, p. 144*): "As it passes out of the warm sections, around Utah and Salt Lake Valleys, its damage has become less and less until, in quite a number of districts, it is pretty hard to convince the farmers that they have it at all."

Titus (*15, pp. 133–134*): "In no place in Idaho is it known to be doing damage. Weevils are so few in most places that you have to search for them."

This statement was made in 1916, when the weevil was confined to the higher parts of southeastern Idaho.

Reeves et al. (*9, p. 88*): ". . . There is hope also that the pest will not prove equally injurious under all circumstances. It is much less harmful in Europe than in America, owing apparently to climatic and industrial conditions, and it multiplies more slowly and does less damage in the higher altitudes in Utah and Wyoming than in the lower valleys."

In considering the migrations of the adult beetles, the statements below may be quoted.

Reeves et al (*9*) *p. 87*): "There is no evidence that the weevils ever fly for the purpose of seeking fields of alfalfa, either new or previously infested, or to find hibernation quarters. The most plausible theory is that their flight is caused by a rise in temperature, as are many activities of the lower animals. So far as can be learned, this flight is at random. It takes some of the weevils into new fields." And, again (*9, pp. 92–93*): "The heat of the soil is also probably an important cause of that increase in the activity of the adults, called the summer flight, which is greatest during the dry, hot weather beginning in June and ending in August. This flight accounts for the presence of many adults in grassy places and orchards, where they alight and find protection from the heat . . . The summer flight is not a general movement of the weevils from the fields to seek more suitable hibernation places elsewhere. There is no such movement, and virtually all of the weevils spend the winter in the fields."

Very little was found regarding the *minimum* fatal temperature. The remark of Reeves (*9, p. 91*), quoted below, is of importance: "Many weevils die in the fields during zero weather, but milder temperatures seem to have little effect upon them."

One of the first successful control measures was based upon the *maximum* fatal temperature. In connection with a discussion of harrowing after removing the first crop Reeves (*8, p. 128*) states: " . . . Under such circumstances the insects can be destroyed and the crop protected by taking advantage of the fact that a temperature of 120° F. is fatal to the insects. This temperature is best produced by covering the surface of the field with something approaching a dust mulch, unshaded by clods and vegetation. On a bright, warm day such a surface is heated by the sun enough to kill all stages of the weevil, and the dust kills many of those which escape the heat."

The very high temperature obtained at the soil surface in direct sunlight has rarely been utilized to control insects.

HUMIDITY

In addition to those passages quoted above, in which reference is made to humidity in connection with temperature, there are a few places in which humidity seems to be more important than temperature.

Titus (*12, p. 49*) says: "During the period when they are going into hibernation they are quite susceptible to climatic conditions; even the passing of the sun behind a cloud will drive them to shelter, and a cold rain following a very hot day will so chill those that have not been able to get into proper shelter that many will not survive the night."

INDIRECT INFLUENCES OF CLIMATIC FACTORS

The parasites of the alfalfa weevil are of two kinds—insect parasites and fungi. The parasitic insects which have proven of importance in the control of *Phytonomus posticus* have been secured from Italy, a region of nearly optimum conditions for the weevil. On the other hand, fungi have been of importance in the Old World near the distributional limits. No information of value has been published regarding the climatic relations of insect parasites, but there are several statements concerning the fungi.

There are two entomophagous fungi reported as attacking the weevil. One of these, *Empusa sphaerosperma*, is an important enemy of a closely related species, the clover leaf-weevil (*Hypera punctata*), in the eastern United States and has killed as high as 44 per cent of the mature larvae of *Phytonomus posticus* at Salt Lake City. (*18, p. 41.*) Webster reports a mortality of about one-fifth on June 13, 1911, at Salt Lake City. The meteorological records show that the spring of 1911 at that place was nearly normal (see table on p. 480), which proves that this fungus is capable of destroying large numbers of the weevils under average Utah conditions and may prove of great importance near the limits of the range, where the spring conditions are cooler and wetter. A second species (*Sporotrichum globuliferum*) has been reported by Rockwood (*10, p. 499*) to be working on the adult beetles in Utah. Some of his conclusions are quoted below:

"The entomogenous fungus *Sporotrichum globuliferum* Speg. develops spontaneously as an infectious disease of the alfalfa weevil, *Hypera variabilis* (*posticus*), on the bench lands of the Salt Lake Valley in the early spring. Infection experiments show the weevil to be very susceptible to fungus infection at this season, a complete mortality from the fungus being secured in breeding cages in usually two weeks time. The ground-frequenting habits of the alfalfa weevil at this season render it particularly liable to infection from contact with fungus-covered insects.

"The new generation of weevils is less susceptible to the fungus during the periods of aestivation and hibernation in the summer and fall. Moreover, favorable conditions for the growth and spread of the fungus are unlikely to occur in Utah at this time.

"The period of greatest mortality from the fungous disease, coinciding as it does with a period of great potential injury from the pest, namely, the oviposition period, makes the fungus worthy of record as a natural enemy of the alfalfa weevil."

Some further remarks by Rockwood (*10, p. 493–494*) on the relations of climate to the abundance of Sporotrichum are relevant: "The fungus *Sporotrichum globuliferum* Speg. was first found on the alfalfa weevil near Salt Lake City, Utah, on March 14, 1914. It was frequently met with and could easily be found on weevils and other insects under alfalfa plants from that time until May. This was a time of considerable precipitation and the ground in alfalfa fields was moist to wet most of the time. The time of greatest abundance of the fungus was April 21 to 29. At this time at least one weevil killed by the fungus could be found under almost every plant examined . . . . . In the early spring, the optimum conditions for the growth of the fungus are likely to be found in all the alfalfa fields on the East Bench of old Lake Bonneville regardless of the irrigation practice.

"Later in the season when the spring rains have ceased, the fungus seems to be restricted to fields which are generously irrigated and have a heavy, close stand of alfalfa. One such field was examined on July 29 and 28 weevils with a pure growth of Sporotrichum were picked up in a short time. Such mortality at this late date is of slight importance, however, as this is the time when the overwintered adults are dying off naturally. Yet it is worthy of note that this field for which a generous water supply was available and which was therefore lavishly irrigated has never been seriously injured by the weevil, at least so the rancher informed me, and I was inclined to believe him, as it was certainly unusual for a Utah farmer to deny injury from the weevil . . ."

The above statements give a basis for determining the optimum conditions for fungous growth on the alfalfa weevil at Salt Lake City. The normal temperature for April is 50° F., and the normal April rainfall about 2 inches. So far as the production of a favorable humidity is concerned, this would be roughly equivalent to a monthly rainfall of 2½ inches at 60° F., or of 3 inches at 70° F. (*2, p. 65*). On this assumption, we can estimate the climatic suitability of any region for fungous growth by studying its approach to this curve, and we can tell whether or not the fungus *Sporotrichum globuliferum* will be an economic factor. This point is used frequently in studing the distribution of the alfalfa weevil in America.

Summary of Climatic Relations of Phytonomus Posticus in America

The following points of importance in this study have been found in this survey of American literature:

The limiting temperatures are about 0° F. and 120° F.

Warm, dry spring weather is essential for the rapid multiplication of the weevil. Such weather shortens the season of oviposition and produces a maximum number of larvae working simultaneously.

The damage decreases with an increase in altitude. An increase in altitude in this region is equivalent to a shortening of the growing season plus an increase in precipitation.

Cold, damp spring weather like that of Montana, as opposed to the hot, dry spring weather of the Great Basin, not only retards the rate of development of the weevil itself, but is favorable to the increase of fungous enemies, which aid in checking the damage.

THE CLIMATIC ECOLOGY OF PHYTONOMUS POSTICUS IN THE OLD WORLD

The Old World distribution of the alfalfa weevil is given by Titus (*13*) as all of Europe, southern Siberia, Turkestan, Asia Minor, Persia, Arabia, north coast of Africa, Madeira, and Canary Islands. He records outbreaks of this or some very closely related species in southern France, Italy, and southern Russia.

This is a very general statement from which to develop the climatic relations of this insect, so the writer has used a second method of determining the economic distribution. A search was instituted through two abstracting periodicals, the Review of Applied Entomology, Series A, and the International Review of the Science and Practice of Agriculture. The material is presented below in three categories. "Mention" indicates a mere mention of the insect in a general list of pests, with nothing to show that any damage was done by it. "Minor pest" indicates that some slight damage was done in the year indicated. "Major pest" indicates a serious outbreak, recorded in one or several extended articles. Many of the articles recorded below are not important in the further study of the weevil, so the original sources are not quoted.

Economic Distribution of P. Posticus

MAJOR PEST:

Southern France.—1914. Controlled to some extent by a fungus, *Entomophthora sphaerosperma.*

Italy.—No recent references. Titus (*13*) gives the following dates of outbreaks: 1884, 1890, 1909, 1910, 1911.

Turkestan.—1913. In connection with a paper on the utility of the Wagtail, an insectivorous bird, the alfalfa weevil is noted as a very serious pest. It does a large amount of damage, there being no natural control for it except birds.

———. 1914. Listed as a major pest in the valley of Isfara (Ferghana) this year.

MINOR PEST:

Sweden.—1912.

Denmark.—1916. Districts of Lolland-Forster and Naestvod, controlled by fungus.

Germany.—1922.

Southern Russia.—Governments of Kiev, Moscow, and Kherson. No dates given.

Transcaucasia.—Districts of Tiflis and Erivan. Was a pest in these regions in 1916–17.

MENTION:

Denmark.—1918, 1919, 1920.

Russia.—Moscow. No dates given.

Astrachan.—Mentioned in a list of pests of alfalfa.

Reeves (*8, p. 130*) makes the following statement, which explains the scarcity of serious outbreaks in Italy. . . . "There is a possibility that in a different climate from that of Utah

the weevils would not be forced into inactivity by the heat of summer, the generations would be more or less spread out, and the feeding would continue through a larger portion of the year but be less concentrated and therefore less destructive. . . . Mr. H. S. Smith, who studied the insect in Italy, was of the opinion that that was one of the principal factors in producing the condition in Italy along the seacoast, where the weevil is always present in considerable numbers, but is of no consequence as a pest. Its feeding is distributed through many months instead of a few weeks."

Summarizing the above information: The alfalfa weevil is at times a major pest in southern France, Italy, and Turkestan; a minor pest in Germany, Sweden, Denmark, southern Russia, and Transcaucasia. If that gives an approximate picture of the economic distribution of this species, the climatic optima are found in the Mediterranean region and Turkestan and the limiting conditions farther north, in Germany, Sweden, Denmark, and southern Russia, with a limiting condition of a similar nature in Transcaucasia.

Climatic Conditions

The writer has spent considerable time collecting and comparing the climatic data for the various regions listed above, and is greatly indebted to C. F. Talman, librarian of the United States Weather Bureau at Washington, for securing data and for other services in this connection. The basic data have been taken from Hann (*3*) and Kendrew (*4*), and it is impracticable to cite specific references for each quotation. The climatic data have been very carefully studied, and no attempt is made to give those for any given station in full, such a citation being replaced by a summary of the important conditions of a general region as shown by a study of several stations.

Optimum Conditions

Mediterranean region.—The general character of the annual cycle of temperature and precipitation is well shown in the climograph for Marseilles, France (fig. 1). The climograph of Ball is a graph on which monthly means of temperature are plotted against monthly totals of precipitation. The points are joined in chronological order, so that a picture of the annual cycle of temperature and rainfall is obtained. All the stations studied in southern France and Italy have essentially similar climates, so that the region may be treated as a unit. The important factors in this study are (1) the warm, wet winters, no month having a mean temperature below freezing, and (2) the hot, dry summers, seven to nine months being above 50° F. and five to six months above 60° F. in mean temperature. The spring from April to July is increasingly dry, March and April being the only months favorable for fungous growth. The precipitation of the three summer months—June, July, and August—ranges from 2½ to 4 inches for the period, averaging very close to 3 inches. The mean relative humidity in summer ranges from 50 to 65 per cent, and all times of the year are very sunny, most of the winter rainfall coming in heavy showers, followed by bright sunshine.

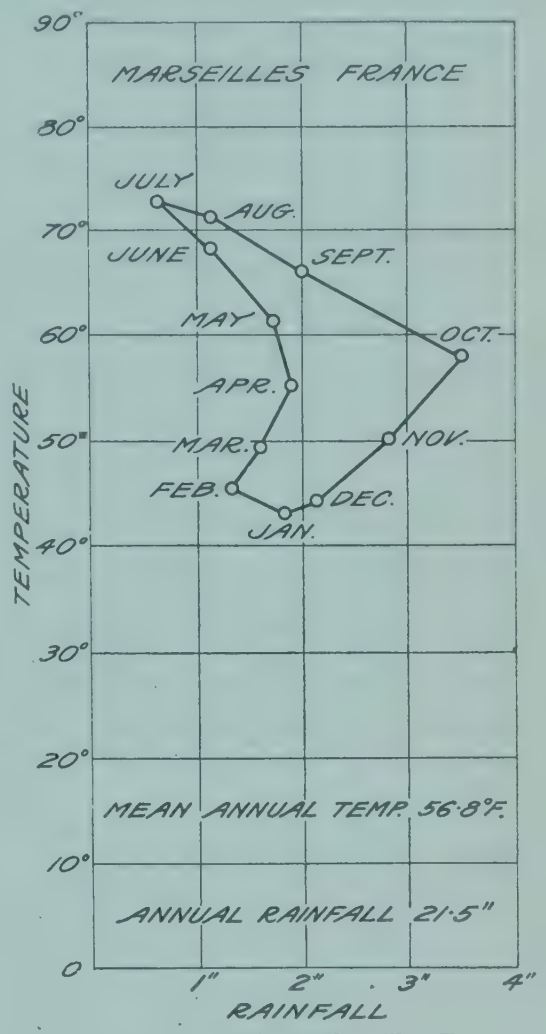

Fig. 1.—Climograph for Marseilles, France

Turkestan.—The attacks of the weevil in this region have been most severe in the Valley of Ferghana. This is an irrigated valley in the mountains of the southern part of the province, which has climatic conditions very comparable to those of

the Salt Lake Valley in Utah, and for that reason a study of its climate is of considerable interest.

At the station of Tashkent (fig. 2) a mean annual temperature of 56° F. is found, with five months above 60° F. and seven months above 50° F. The mean annual maximum is 103° F. and the mean annual minimum −4° F., the absolute minimum for 30 years being −19° F. The rainfall comes largely in early spring, March and April being the wettest months, with conditions very favorable for fungous growths. The spring is increasingly dry until July, and the total rainfall for the summer months is only 1 inch. To judge from the summer temperatures, the frostless season must be well over 150 days. The relative humidity is 63 per cent for the annual mean and 48 per cent in July. There is sufficient winter precipitation to provide a snow cover at the times of subzero temperatures.

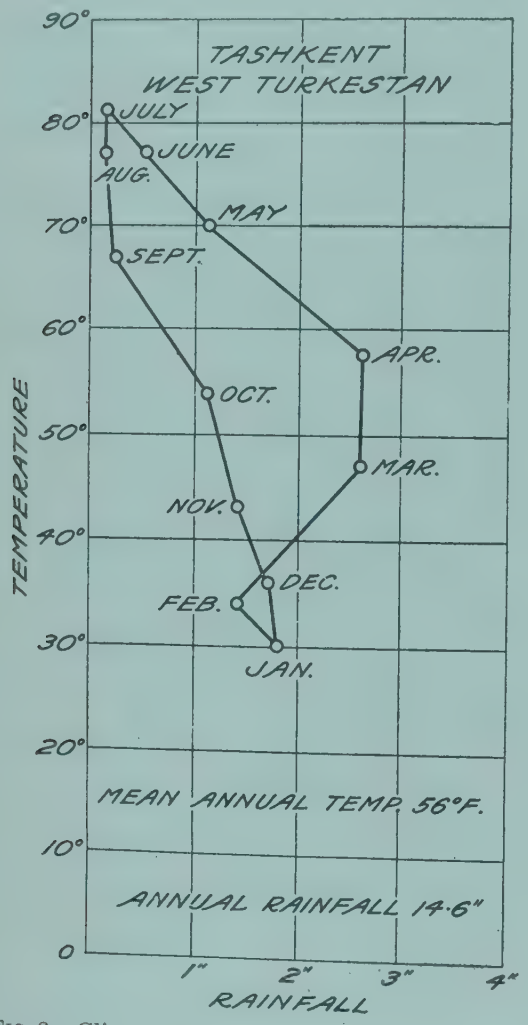

FIG. 2.—Climograph for Tashkent, West Turkestan

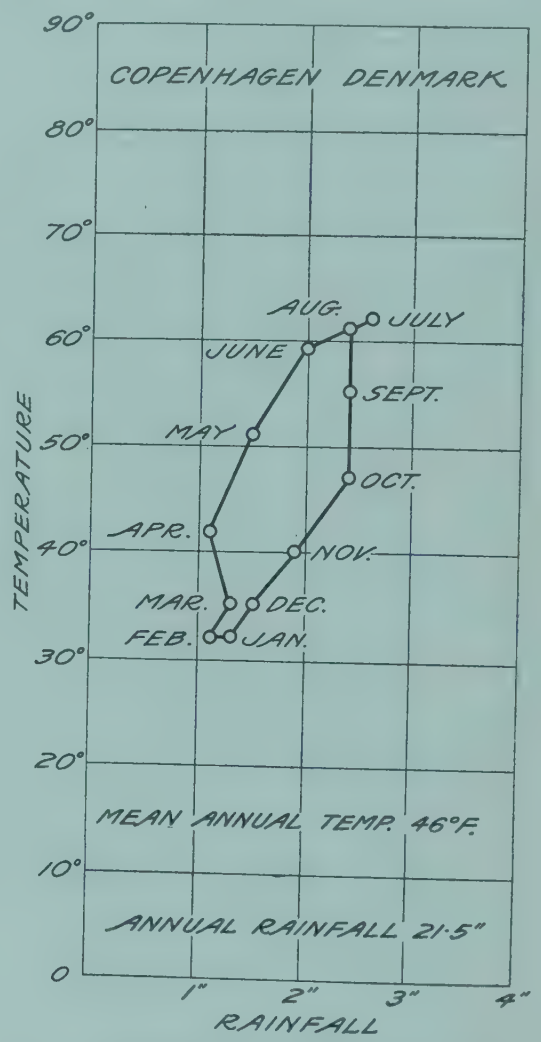

FIG. 3.—Climograph for Copenhagen, Denmark

LIMITING CONDITIONS

SCANDINAVIAN COUNTRIES.—Copenhagen, Denmark (fig. 3), has been chosen as typical of conditions in this general region. The climate is maritime, with fairly evenly distributed rainfall. The spring is the driest part of the year, with the rainfall gradually increasing to a maximum in July, when conditions become favorable for fungous growth on the alfalfa weevil. The summer, like the spring, is cool, so that feeding by the weevil must be scattered over a long time. A reference in the Review of Applied Entomology notes larval feeding in July (*6*). This would indicate that growth is retarded. Fungous enemies are also noted. Thus the limiting conditions in this region are cool summers and too abundant moisture.

SOUTHERN RUSSIA.—Of the three governments listed in the table, that of Moscow is farthest north and should furnish a better index of limiting conditions than either of the others. The data from several stations in Kiev and Kherson indicate that heavy spring rainfall is the limiting factor. The climograph for Moscow (fig. 4) shows a climate quite similar to that of our western plains, although the winter

minimum of rainfall is not so strongly marked. The limiting factors appear to be summer rainfall, which averages about 7.6 inches, and winter temperatures. The mean annual minimum temperature is —23° F., and the five winter months average below the freezing point. The growing season is about 165 days. The spring is slow, so that feeding is distributed over a long period.

ASTRACHAN.—The climograph for Astrachan is shown in Figure 5. The climate is warmer than that of Moscow,

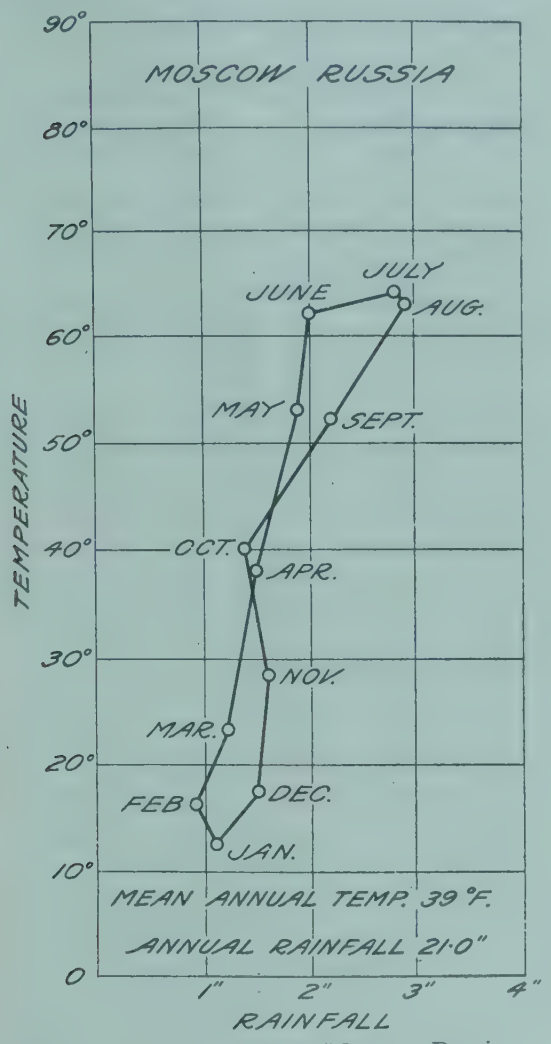

FIG. 4.—Climograph for Moscow, Russia

and the rainfall at all times is very light, so that the limiting factor in this case must be winter temperature. The mean annual minimum is —13° F., and what little snowfall there is must be swept away by the winds, so that the weevils are kept down by the cold winters. Spring and summer conditions of temperature and precipitation are close to the optimum, being warm and dry.

TRANSCAUCASIA.—This is a mountain region of extremely varied climate. Data are available from the two stations of Tiflis and Erivan, and their climographs are reproduced in Figures 6 and 7. Tiflis has warmer winters than Erivan, but Erivan has drier summers. The limiting condition at Tiflis is apparently the precipitation in the spring months, May, especially, being favorable to fungous enemies. At Erivan summer conditions approach more closely to those of the Mediterranean region, but the winters are cold.

No data are available regarding minimum temperatures at Erivan, but the absolute minimum at Tiflis is 1° F.,

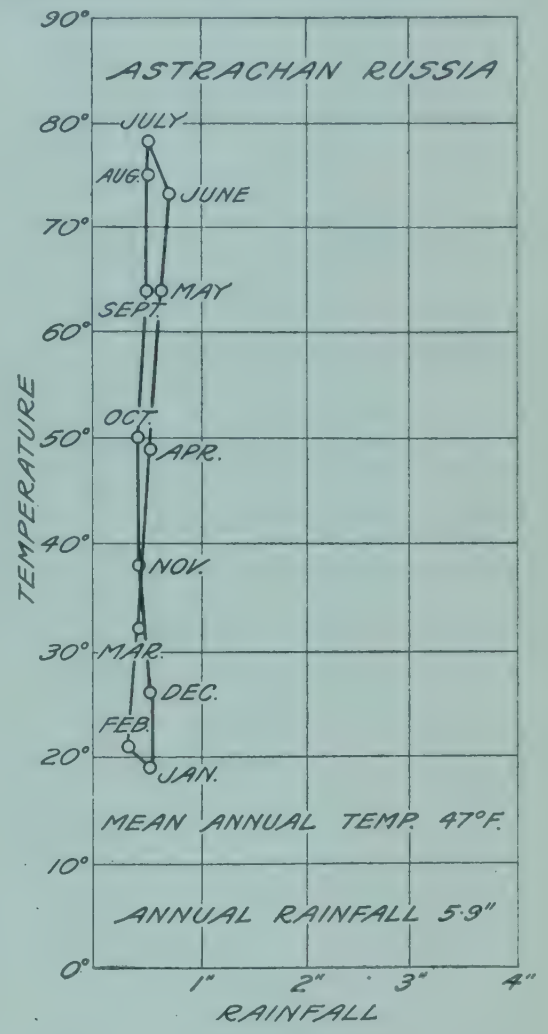

FIG. 5.—Climograph for Astrachan, Russia

and the winter months at Erivan average 10° F. colder than at Tiflis, so that subzero temperatures must be fairly common. Of the two regions, that around Erivan approaches the rainfall optimum, whereas Tiflis presents a limiting condition of rainfall.

SUMMARY OF THE CLIMATIC RELATIONS OF PHYTONOMUS POSTICUS IN THE OLD WORLD

The climatic requirements of the alfalfa weevil in the Old World will

here be summarized for comparison with those requirements in America.

One of the limiting conditions is winter temperature. The insects are killed by exposure to 0° F., and places which normally have subzero temperatures, not accompanied by heavy snow covering, are shown to be found on the limits of distribution. (Moscow, Astrachan.)

Warm, dry spring weather is shown to be essential to the rapid development of the weevil. Such conditions obtain in southern France, Italy, and Turkestan, and to a lesser extent in Tiflis. The spring must be increasingly dry, and the summer precipitation must be not more than 4 inches. Each of the months whose mean temperature is above 50° F. should have less than 2 inches of rainfall.

Damage decreases with an increase in altitude. This is climatically equivalent to an increase in latitude. The northern boundary is found in regions having a growing season of about 150 days, with a mean annual temperature of about 40° F., four months above 50° F. and three months above 60° F.

Cold, damp spring weather favors the increase of fungous enemies. Such weather is found in Denmark, southern Russia, and to a lesser extent in Transcaucasia. Months whose mean temperature is above 50° F. have more than 2 inches of rainfall.

No data are available from which to determine the factors which limit distribution on the south.

For the purpose of applying these findings to American conditions they are condensed into the following conditions:

(*A*) Optima:
- Mean annual temperature 50° F. or above.
- Summer rainfall not over 4 inches.
- No winter temperatures below 0° F. or, if below, in combination with heavy snowfall.
- Growing season of 150 days or more.

(*B*) Limits:
- Mean annual temperature below 40° F.
- Summer rainfall over 6 inches.
- Subzero winter temperatures combined with light snowfall.
- Growing season less than 100 days.

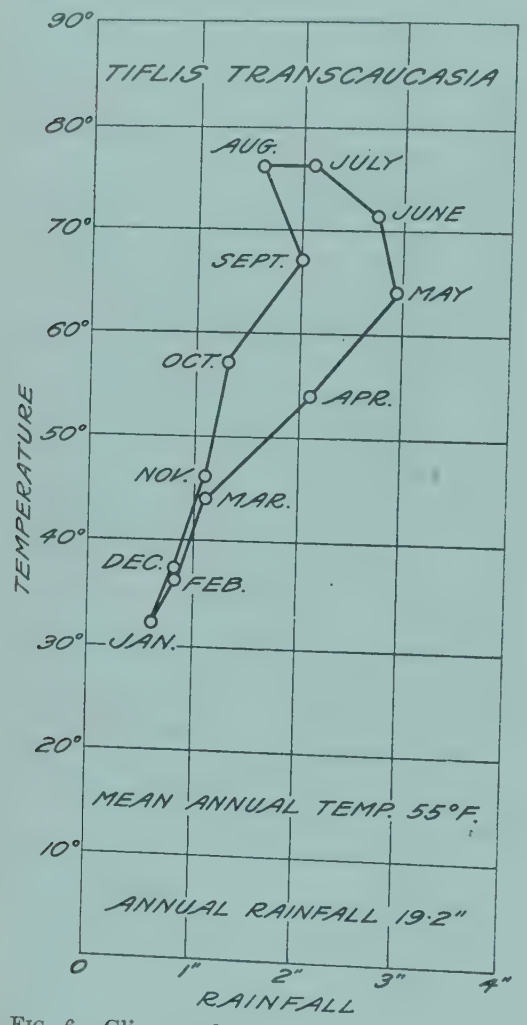

FIG. 6.—Climograph for Tiflis, Transcaucasia

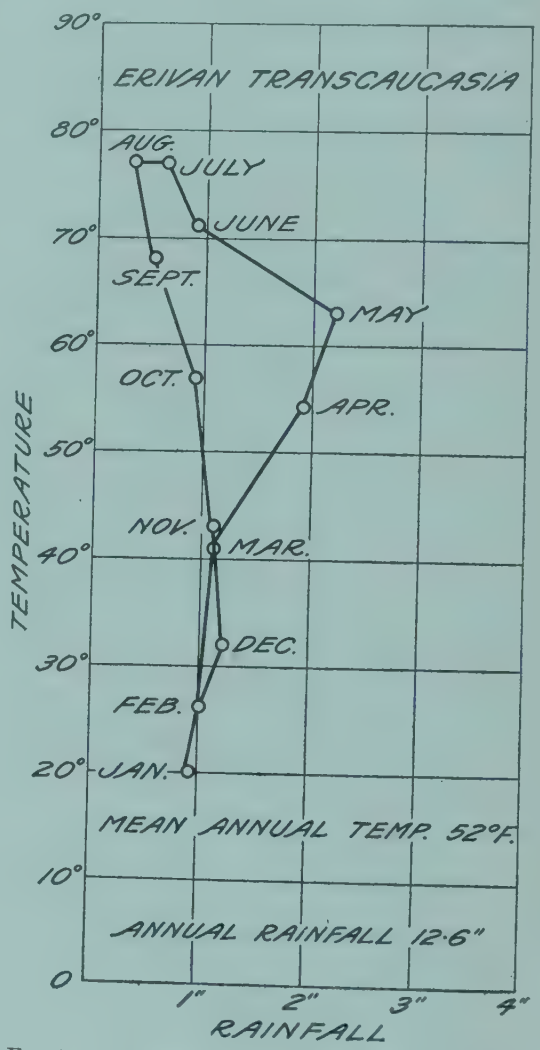

FIG. 7.—Climograph for Erivan, Transcaucasia

SUITABILITY OF AMERICAN CLIMATE FOR THE ALFALFA WEEVIL

Rainfall

Students of American climatology recognize several types of rainfall distribution. Ward (*17*) lists 14 rainfall types, but for our purpose there are 6 main types, as designated by Kincer (*5*), which he calls the Florida, Eastern, Plains, Arizona, sub-Pacific, and Pacific types.

Of these 6 types the summer rainfall is less than 6 inches in four types and less than 4 inches in two of these. The Plains type is characterized by a summer maximum, the summer rainfall ranging up to 12 inches or more. However, there are certain parts of the plains, on the eastern slope of the Rockies, in which the summer rainfall is from 4 to 6 inches, and it is in these places that the weevil may possibly occur. The Arizona rainfall type is characterized by a late summer maximum in July and August, but there are some regions in this type in which the summer rainfall is very small. The Pacific type has a very pronounced winter maximum, with almost rainless summers. The sub-Pacific type, illustrated by Salt Lake City (fig. 8), has no pronounced winter maximum, but fairly heavy rainfall occurs from March to May. Farther north in Idaho there is a more pronounced winter maximum, with a secondary maximum in May. (Payette, fig. 9.) In western Colorado the rainfall is very light with no pronounced maximum. (Delta, fig. 10.)

The weevil is apparently excluded from most of America east of the Rocky Mountains by abundant spring and summer rainfall. It would appear to be limited to such districts west of the Rockies as have favorable temperature conditions and to a small area lying immediately east of the Rockies, which has scanty summer rainfall.

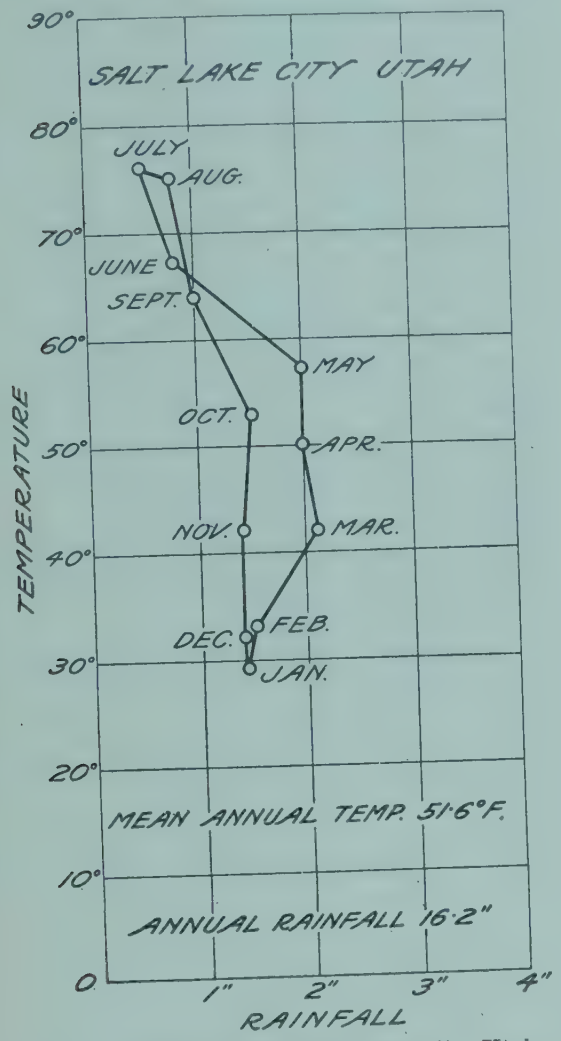

Fig. 8.—Climograph for Salt Lake City, Utah

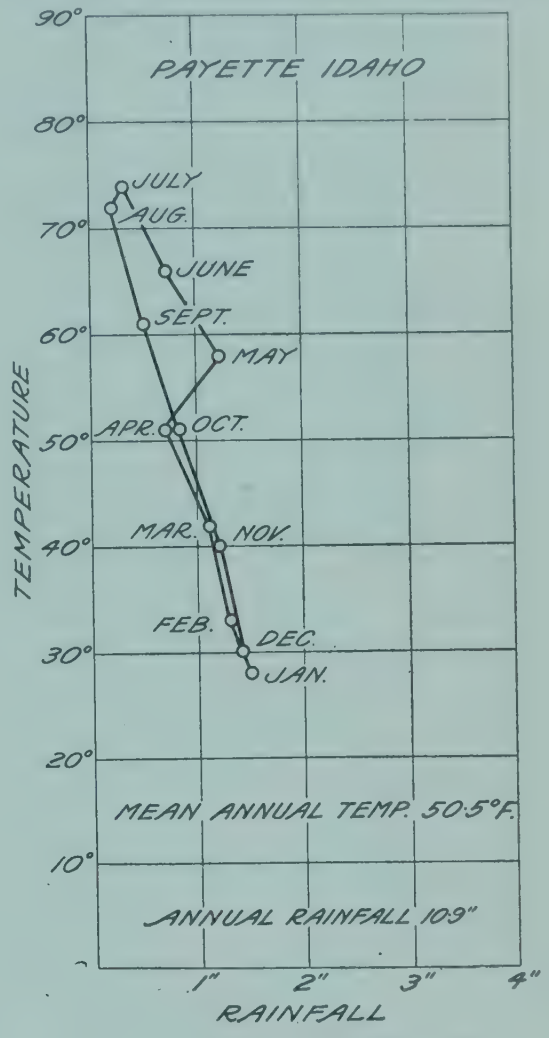

Fig. 9.—Climograph for Payette, Idaho

Temperature

Within the rainfall regions outlined above the distribution and abundance of the weevil are controlled by temperature. Regions having a mean annual temperature of 50° F. or higher approach the optimum. When this tem-

perature is approached, although winters are cold, the weevil may maintain itself and become of economic importance periodically. A mean annual temperature of 40° F. closely marks the northern limit of economic distribution.

Probable Distribution in America, Based on Climate

The writer carefully analyzed the climatological data from the Western States, considering all the possible

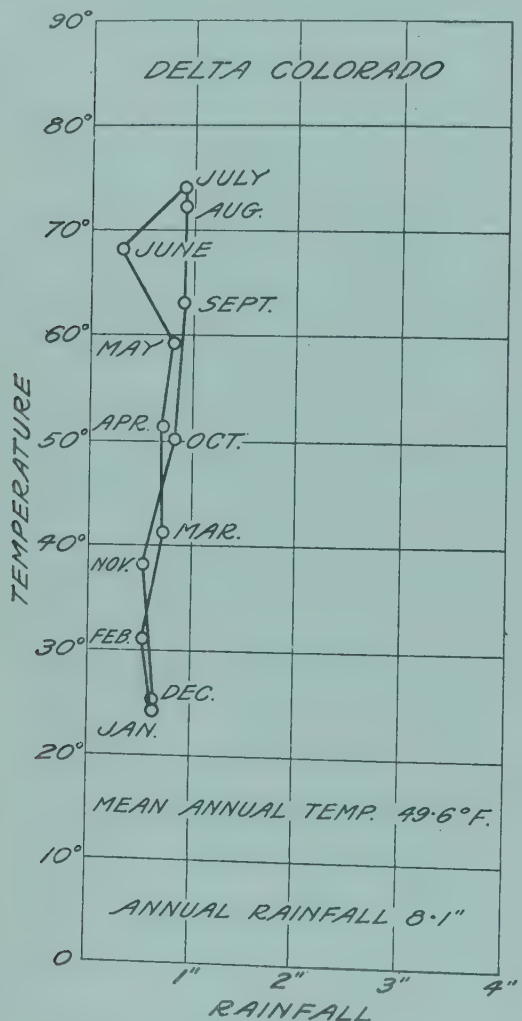

Fig. 10.—Climograph for Delta, Colo.

combinations of temperature and rainfall found to be of importance in the ecology of the alfalfa weevil. The stations may be tentatively grouped under three categories:

1. Regions whose normal climate approaches the optimum for the alfalfa weevil may be regarded as subject to severe infestation and included in an area of "normal" occurrence.

2. Regions whose normal climate departs slightly from the optimum may be regarded as subject to periodic infestation in times when the climatic variations are toward the optimum. These regions are areas of "occasional" occurrence. The weevil is able to maintain itself at all times in these areas and becomes abundant when conditions become favorable.

3. Regions whose normal climate varies widely from the optimum, in which optimum conditions will rarely occur, may be included in an area of "possible" occurrence. The weevil will probably not maintain itself in this area but may be repeatedly introduced and become of minor importance after a series of favorable seasons.

The probable distribution of the alfalfa weevil in America, based upon these climatic requirements, is shown in the map (fig. 11). The normal occurrence of the weevil is limited entirely to the sub-Pacific and Pacific rainfall regions, which are indicated on the map. There are two localities outside of these regions which are included in the second zone, one in northern Wyoming and the other in western New Mexico. Rainfall conditions are favorable in Wyoming, but the mean temperature is low and the winters are cold and dry; therefore this area is not of great importance. Western New Mexico, on the other hand, has favorable temperature conditions, but the rainfall is of such a character that wet years are very likely to occur often enough to keep the insect in control. The possible occurrence of the outer zone, which are confined to the Plains and Arizona rainfall types are chiefly conditions comparable to Moscow, Russia (fig. 4), but with more extreme winter conditions.

Broadly speaking, the alfalfa weevil is limited on the north, inside the Pacific and sub-Pacific rainfall regions, by low summer temperatures; on the east, at the edge of the sub-Pacific type, by heavy summer rainfall; and on the northeast, in the plains of Montana and Wyoming, by the summer rainfall and by low winter temperatures, plus a very light snowfall.

No data have been found upon which to base a limit on the south, but it is possible that the high temperatures of southern California and Arizona may prove fatal to larvae. All of this region is included in the normal zone in this study, and must remain so until the factor controlling southern distribution becomes evident.

The question of the possible adaptation of the alfalfa weevil to new climatic conditions in America has not been approached in this paper and does not seem of importance to the writer. The weevil is a native of Europe and

has been limited on the north by purely climatic factors for a long period of time. There is no physiographic barrier at its northern limit in Russia and Siberia, and it seems probable that it has reached its limit of adaptability by the present time, so that little or no further adaptation to cold and moisture will take place.

persal of the weevil by approximately five-year periods. This map does not show the present distribution accurately, as a county has been recorded as infested when weevils have been found in any portion of it. The infestation in southern Wyoming, for example, does not cover more than a third of the area shown in Figure 12.

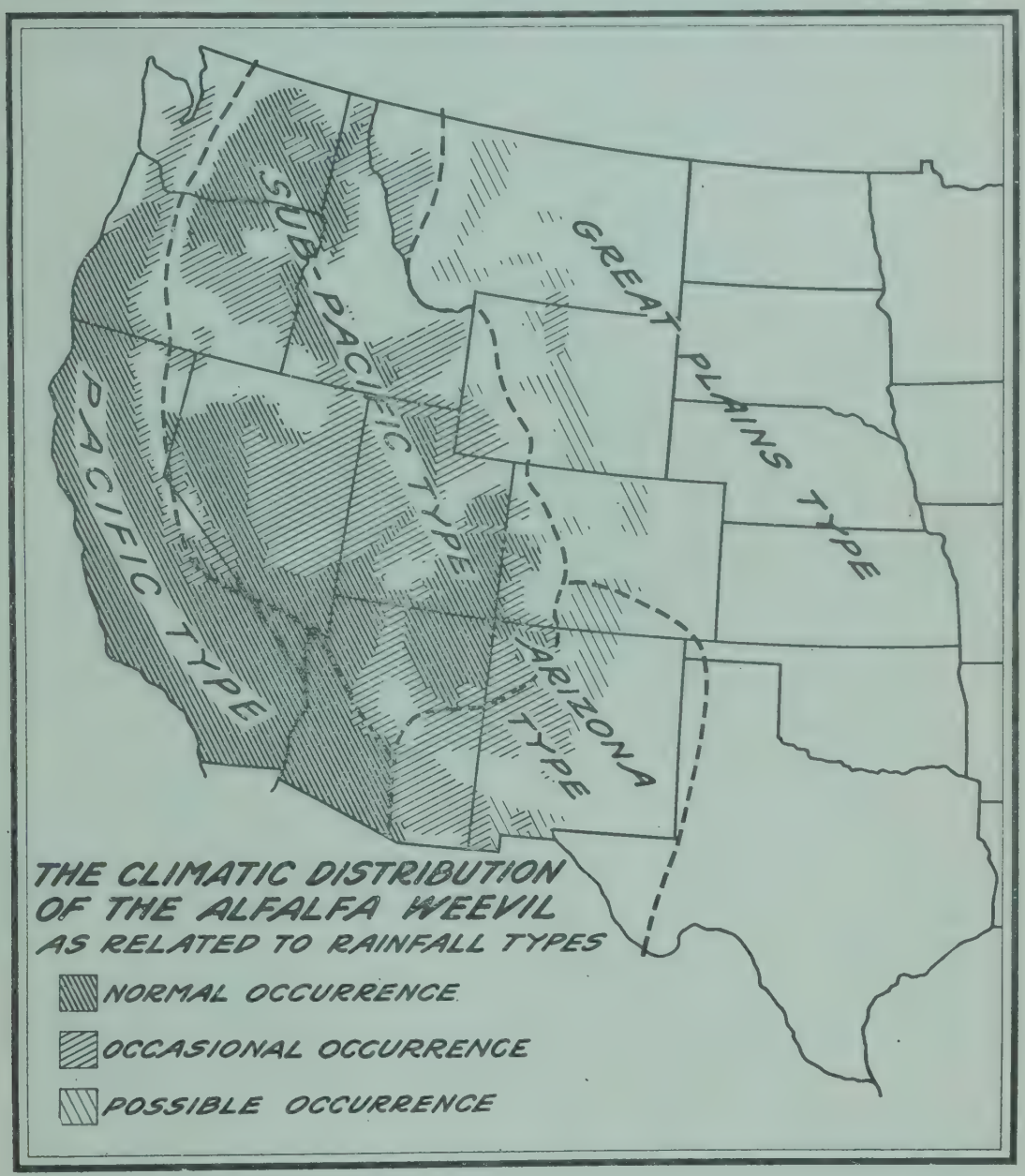

FIG. 11.—Probable distribution of the alfalfa weevil in America, based on climatic limitations

PRESENT DISTRIBUTION

Through the kindness of George I. Reeves, of the United States Bureau of Entomology, the writer obtained a detailed statement of the annual spread of the alfalfa weevil by counties since its introduction and discovery at Salt Lake City in 1904. This information has been summarized and a map prepared (fig. 12), which shows the dis-

This map brings out several interesting facts. All regions infested at the present time are within the rainfall area of the sub-Pacific type. The weevil is still spreading northward and westward in Idaho and Oregon and southward in Utah. On the other hand, it reached its climatic limits in Colorado in 1917, and its dispersion has been only local in that region since that time. It has not crossed the

Continental Divide there. It has been present in southwestern Wyoming for eight years and has not crossed the climatic limit shown on the map. It reached the northeastern corner of Idaho in 1917, and has not yet crossed the climatic boundary into Montana.

Within the present range the regions of the most severe infestation are the lower valleys of Utah, the Colorado area, western Idaho and the adjacent part of Oregon, and western Nevada. It has been abundant in some of the other places, but varies greatly in abundance from year to year. In short, it is most abundant in the zone of "normal" occurrence, is often injurious in the zone of "occasional" occurrence, but has not as yet reached the zone of "possible" occurrence. It is spreading at the present time in those parts of its range where conditions are favorable from a climatic standpoint and has ceased to spread when it has reached the climatic boundaries. Thus the history of the insect in America substantiates the findings of the studies on climatic conditions in the Old World.

The zones outlined on the map of Figure 11 are based upon a detailed study of the climatological data from several hundred stations in this region as published by the United States Weather Bureau.[3] The writer wishes to express appreciation of the assistance rendered by the section directors of the Weather Bureau for these Western States in furnishing a large amount of climatic data and in some cases lending file copies of the older publications.

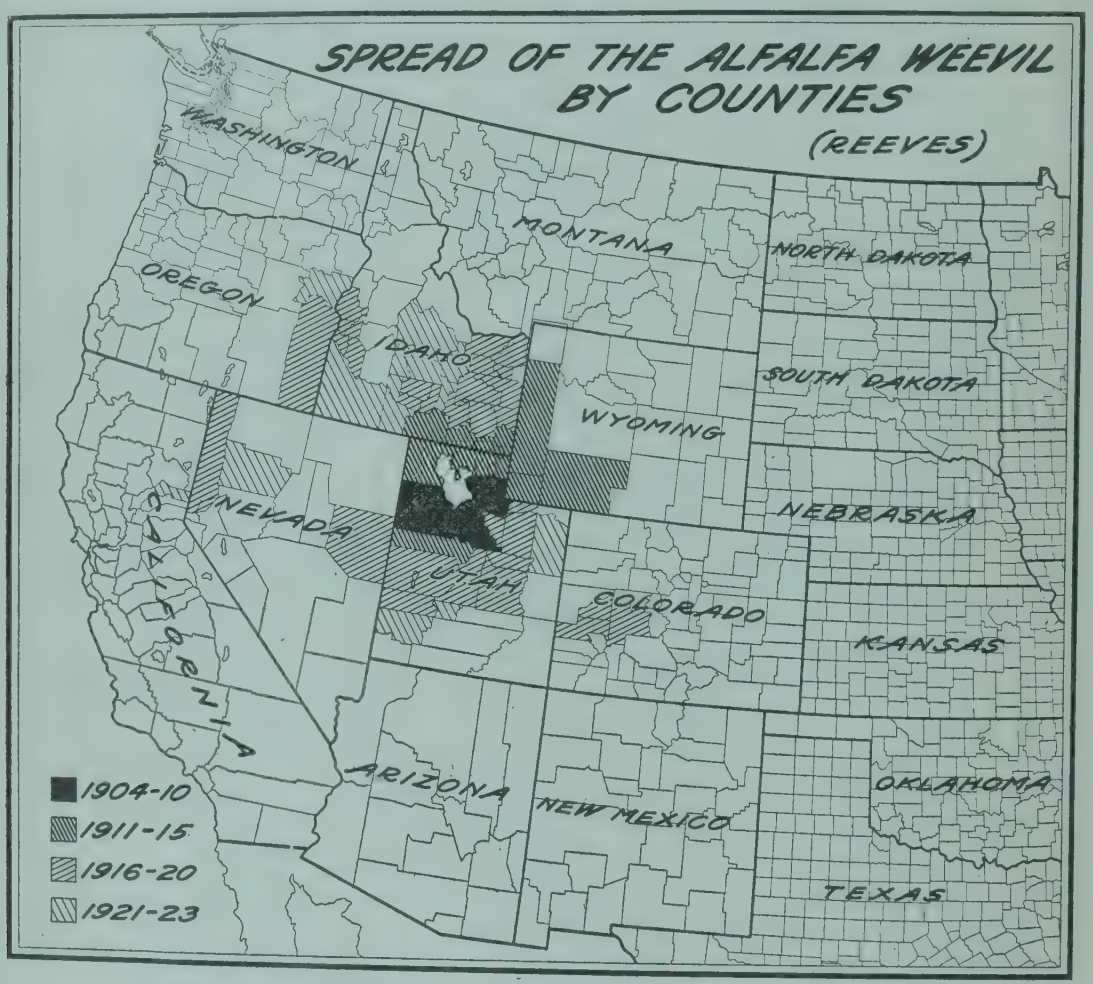

FIG. 12.—Dispersion of the alfalfa weevil in America, 1904–1923

SUMMARY AND CONCLUSIONS

The general procedure indicated in the introduction has been used in the present paper.

The climatic conditions of the original home of *Phytonomus posticus* have been studied in detail to find the optimum and limiting conditions, which were correlated with similar conditions in America.

[3] UNITED STATES DEPARTMENT OF AGRICULTURE, WEATHER BUREAU. SUMMARY OF THE CLIMATOLOGICAL DATA FOR THE UNITED STATES, BY SECTIONS. 106 sections, published separately. 1908 and later dates.

UNITED STATES DEPARTMENT OF AGRICULTURE, WEATHER BUREAU. CLIMATOLOGICAL DATA BY SECTIONS. Annual summaries under this general title for various Western States. 1910–1922.

Climatic conditions in America were analyzed to determine the areas of optimum and limiting conditions, which have been charted and compared with the present distribution of the alfalfa weevil. They have been found to correspond in all essential particulars with the progress of the present infestation.

It seems highly probable that the alfalfa weevil has been imported into this country many times and that it also has often been shipped out of the quarantined areas into other parts of the country, but that it has failed to establish itself except where it was placed in a region of favorable climatic conditions.

The climatic limitations of other insects are different, for every insect has its own definite optimum and limiting factors. Some insects, like the San Jose scale and the brown-tail moth (*1*) are definitely limited on the north by minimum temperatures. Others require a certain length of growing season. Still others, chiefly boreal forms, can endure intense cold but succumb to heat and have a definite southern limit. With regard to rainfall, the same is true. Insects like the army worm and the variegated cutworm require a humid condition, and others like the pale western cutworm require a dry condition. It seems highly probable that there are one or more periods in the life of any insect when it is especially susceptible to unfavorable climatic conditions. If these periods can be found, and their climatic optima and limits determined, the range of the insect can be mapped. In the case of the alfalfa weevil the limiting periods are two in number—the temperature limit, which applies largely to the hibernating adults, and the humidity limit, which applies to the larva and its fungous enemies.

The method of climatic analysis used in this paper has been applied successfully to an insect inhabiting the soil (the pale western cutworm (*2*)) and to an insect feeding on the aerial portions of low plants (the alfalfa weevil). In order to determine the applicability of the method to insects in general, it remains to apply it to some insect living in trees. The forest environment, either natural or in a cultivated orchard or grove, is widely different from conditions in the open, and the problem must be attacked by an analysis of actual forest conditions, after which these conditions must be correlated with the available weather data from near-by stations. For this reason the method will probably need considerable modification before it will apply to forest insects.

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INFECTION AND DISSEMINATION EXPERIMENTS WITH DEGENERATION DISEASES OF POTATOES. OBSERVATIONS IN 1923[1]

By E. S. SCHULTZ, *Pathologist, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture, and* DONALD FOLSOM, *Plant Pathologist, Maine Agricultural Experiment Station.*

INTRODUCTION

In a former publication[2] evidence was presented indicating that degeneration of the potato (*Solanum tuberosum* L.) is brought about by at least seven diseases, for which the etiology remains to be demonstrated. It was established that although these diseases are quite similar regarding transmission, they differ sufficiently in transmission and symptoms to be considered distinct, even in the same variety and in the same environment. Furthermore, it was shown that combinations of these diseases occur, usually causing more severe injury than a single disease. Insect transmissibility was proved for six diseases and for several combinations.

These results suggested that further investigations might disclose additional similar maladies; that some unsolved apparent combinations might be reduced to still simpler forms; that further knowledge of the reactions of different varieties to those single diseases and their combinations was desirable; that additional information on insect carriers, including different genera or species of aphids and other insects, deserved further attention; and that additional data on alternate hosts should be obtained. In order to secure further evidence on these and similar problems relating more or less directly to control, the investigations presented in this paper were conducted.

DESCRIPTION OF SYMPTOMS

The symptoms of the different diseases to be considered will now be indicated. Four types of mosaic, mild, leaf-rolling, rugose, and crinkle mosaic, are recognized. Mild mosaic (pl. 6, A, 3; B, 4 and pl. 7, A, 2) is characterized chiefly by distinct mottling, wrinkling, some ruffling, and slight dwarfing in the foliage, and by reduction in the yield rate of tubers without modification of their shape or color. Infection with juice inoculations is more difficult with mild mosaic than with rugose mosaic or streak. Leaf-rolling mosaic (pl. 6, A, 2; B, 2, and pl. 9, C) differs from mild mosaic in its diffuse mottling and in a slight rolling of the middle and upper leaves; it resembles mild mosaic in the difficulty of infection by juice inoculations. Tuber symptoms resemble those of mild mosaic. Leaf-rolling mosaic is distinguished from leaf-roll (pl. 10, A) by the absence of distinct rolling, dwarfing, or rigidity, and of chlorosis. Rugose mosaic (pl. 6, A, 1; B, 1; pl. 7, A, 1; and pl. 8, C and D) is characterized by somewhat diffuse mottling, distinct dwarfing, rugosity, and a shorter life period (pl. 7, A, 1) and in current-season or late previous season infections (pl. 8, C) by brittleness, spotting, and streaking, resulting in abnormally premature death. Tuber reduction is more marked than in mild mosaic. Infection by means of leaf mutilation is easier with rugose mosaic

[1] Received for publication May 20, 1924; issued June, 1925. This paper is based upon investigations conducted as a cooperative project between the Office of Cotton, Truck, and Forage Crop Disease Investigations of the Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Plant Pathology of the Maine Agricultural Experiment Station. Unless otherwise indicated, the work was performed in northeastern Maine. The order of arrangement of the authors' names is not intended to indicate that one cooperating institution contributed more than the other to the results.

[2] SCHULTZ, E. S., and FOLSOM, D. TRANSMISSION, VARIATION, AND CONTROL OF CERTAIN DEGENERATION DISEASES OF IRISH POTATOES. Jour. Agr. Research 25: 43–118, illus., 1923.

than with mild mosaic. What seems to be a fourth type of mosaic, resembles mild mosaic but is characterized by a much greater degree of crinkling, that is, of wrinkling, waviness of the margin, and ruffling. This will be called "crinkle mosaic" (Pl. 6, B, 3). It is not identical or even similar to the "crinkle" of other systems of nomenclature. The latter (crinkle) seems to be nearly identical with rugose mosaic, but in descriptions based upon symptom aggregates in plants of the Green Mountain variety, "crinkle" can not replace the well-known term mosaic, and furthermore rugose mosaic is characterized by less crinkling than some other types of mosaic, at least in the variety and environment most familiar to the writers.

The distinguishing characteristics of leaf-roll (Pl. 10, A) include distinct rolling and rigidity, especially of the lower leaves, dwarfing, chlorosis, and some burning. Tubers, usually quite sessile, are reduced in size. They may, in certain varieties and conditions, show net necrosis resulting in spindlingness of the sprouts, especially at the stem end. Spindlingness of the sprouts also may be a symptom without net necrosis (pls. 5 and 7).

Spindle tuber produces spindling stems and upright, somewhat darker green and slightly rugose and dwarfed foliage. Abnormally spindling, spindle-shaped, and usually dwarfed tubers with more or less conspicuous and apparently numerous eyes, are characteristic of this disease. Infection with spindle tuber is obtained with about the same difficulty as with mild or leaf-rolling mosaic.

The characteristic current season symptoms of streak (pls. 2, 3 and 4, A) include streaking, spotting, brittleness, leaf dropping, and premature death of the foliage. The streaking and spotting are more pronounced on the lower leaf surface than the upper, if they appear on the latter. With the exception of faint chlorotic spots marking the initial stages of streaking and spotting, no mottling develops. Second generation reactions of streak appear as severe dwarfing, curling, wrinkling, brittleness, streaking, leaf dropping, and premature death, often preventing tuber development. Brown discolored areas frequently appear near the eyes on infected tubers. Streaks and spots have been observed on the white corollas of inoculated plants of the Green Mountain variety (pl. 3).

Mottled curly dwarf (pls. 4, C, and 5, E) symptoms include distinct dwarfing, spindlingness, curling, wrinkling, slight rolling, brittleness, burning, and somewhat premature death. The tubers show the same symptoms as in spindle tuber, with more dwarfing. This symptom aggregate appears to be a combination of leaf-rolling mosaic and spindle tuber. Unmottled curly dwarf (pl. 1, A, B, and C) is different from mottled curly dwarf in the absence of mottling and in the tubers being more gnarled and cracked. This seems to be a single disease. The tuber cracking may appear, without the spindlingness, as a symptom resulting from current-season inoculation (pl. 1, A to D).

SECOND GENERATION REACTIONS TO INOCULATIONS MADE IN 1922

LEAF MUTILATION INOCULATIONS MADE WITHIN THE GREEN MOUNTAIN VARIETY IN THE FIELD

In the open field in 1922, leaf mutilation inoculations with different types of mosaic and with leaf-roll, spindle tuber, unmottled curly dwarf, and streak, were performed on plants of the Green Mountain, Irish Cobbler, Bliss Triumph, and Rural New Yorker varieties. Either the second or third hill, or both of these hills, in each four-hill tuber unit were inoculated, leaving two or three uninoculated controls in each tuber unit. Single inoculations were made in each series, with the exception of series 38, 58, and 73, in which the second hill in each tuber unit received two applications, and with the exception of series 71, which received three

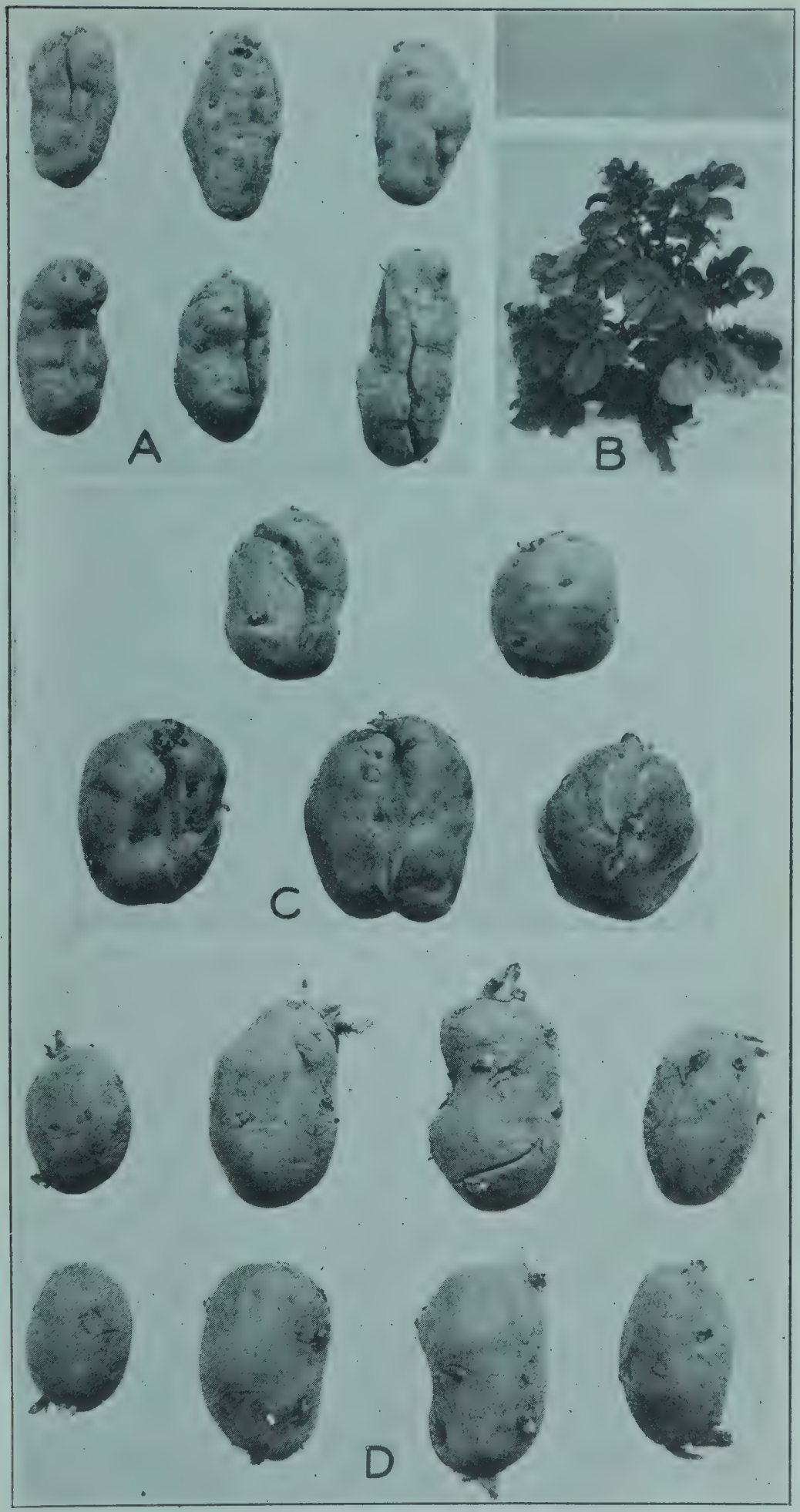
EXPLANATORY LEGEND FOR PLATE 1

A.—Tubers produced by unmottled curly-dwarf Green Mountain plants in a lot serving as the source of inoculum for inoculation series 22 of Table I. For the effects of the inoculation see Plate 1, B, C, and D. Photographed at planting time in 1923

B.—Unmottled curly dwarf plant grown from one of the cracked tubers shown in Plate 1, C. Photographed in August, 1923

C.—Tubers produced by Green Mountain plants inoculated in series 22 of Table I with unmottled curly dwarf, showing cracks as a current-season symptom. Photographed at planting time in 1923. For the complete tuber symptoms see Plate 1, A, for the controls see Plate 1, D, and for the vine symptoms see Plate 1, B

D.—Tubers produced by sister hills of the plants inoculated with unmottled curly dwarf in series 22 of Table I. Four tubers are split and the entire surface of each tuber is shown. One recently formed transverse bruise crack is evident, but is different from the longitudinal healed growth cracks symptomatic of diseased plants (pl. 1, C). Photographed at planting time in 1923



(For explanatory legend see p. 494)

treatments. A number of series showed current season symptoms in 1922 which are noted in a previous paper.[3] Only inoculations performed in the Green Mountain variety are included in Table I.

The disease recorded in the controls was contracted because the plants of the first generation grew close to diseased vines with transmitting insects of at least one species present. Mild mosaic, rugose mosaic, and spindle tuber spread more to the controls than did the other diseases. Inoculations with mild mosaic are not included in Table I, since most of the controls in these series became infected with the same disease. However, the high percentage of mild mosaic observed in the progeny of the inoculated plants in comparison with the percentage in other series, indicates that the inoculation was generally effective. It will be noted that the 1923 progeny of the lots serving as sources of inoculum retained the same symptoms as the 1922 parents, with the exception of the apparent addition of rugose mosaic in series 38 from field infection.

As in the first generation, inoculations with rugose mosaic resulted in a high percentage of disease. The characteristic symptoms of this disease resembled those in the inoculated plants in 1922. Rugose mosaic occurred in combination in series 10, 38, 79, 83, and 127 apparently as a result of inoculation. Another combination inoculation, with leaf-rolling mosaic and spindle tuber, was obtained in series 136. As indicated in a previous paper[3] modifications of symptoms occurred in these combinations. Negative results were obtained with juice from plants having glabrous, more or less crisp and fleshy leaves suggesting mutation,[4] except for the spindle tuber originally in combination. As indicated, in series 95, spindle tuber was associated with the mutation, becoming obvious when the source of inoculum was dug in 1922. In series 22 the tuber cracking of unmottled curly-dwarf appeared as a current-season symptom without the tuber spindlingness, followed by the usual vine and tuber symptoms in the progeny (pl. 1, A, B, C, and D).

As in 1922, streak occurred alone and in combination with rugose mosaic. Perpetuation of streak through the tubers soon ceases because of the lack of progeny. However, a strain of streak from one source has been perpetuated without any apparent combination or change, through four succeeding seasons by means of leaf mutilation inoculation of a new lot each season. Therefore streak is to be regarded as a distinct disease, even though it is closely resembled by a streaking which may occur as a first-season symptom of rugose mosaic, as previously recorded.[5] It is noteworthy that streak alone causes more severe dwarfing in the second generation than results from most combinations of degeneration diseases (pl. 2). The writers have not yet been able to distinguish more than one kind of streak, this one including leaf dropping, spotting of the corollas, and both spotting and streaking of the leaves (pls. 2, 3, and 4, A).

INTERVARIETAL LEAF MUTILATION INOCULATIONS IN THE FIELD

Intervarietal leaf mutilation inoculations were performed in the open field in 1922 with the same procedure and under the same conditions as are described in connection with Table I. Plants with different symptom aggregates in Irish Cobblers, Rural New Yorkers, Bliss Triumphs, and three different seedling lots, served as sources of inoculum for the inoculation of healthy Green Mountain plants. The current-season reactions to these treatments are described in a previous paper.[6] The second generation reactions are given in Table II of the present paper.

Mild mosaic was produced only in series 42, 46, and 54, with Bliss Triumph as the source of inoculum. In series 54 the Bliss Triumph foliage showed marked crinkling in addition to the apparent mild mottling. As indicated, this symptom complex was manifested also in the inoculated Green Mountain plants as crinkle mosaic.

The results with leaf-roll, as in former leaf-mutilation inoculations, were negative. (Series 71.)

The reactions in series 111, 113, 117, 119, and 121 regarding mosaic in the inoculated plants varied from those in the source of inoculum; although mottling could not be detected in the Rural New Yorker plants, it appeared either as the leaf-rolling or rugose mosaic type in the inoculated Green Mountain plants, indicating a varietal

[3] SCHULTZ, E. S., and FOLSOM, D. Op. cit. See Table XVII.
[4] FOLSOM, D. MUTATIONS OF THE POTATO. TWO SOMEWHAT UNSTABLE LEAF-FORM SPORTS OF THE IRISH POTATO. Jour. Heredity 14: 45–48, illus., 1923.
[5] SCHULTZ E. S., and FOLSOM, D. Op. cit. p. 79.
[6] SCHULTZ, E. S., and FOLSOM, D. Op. cit. See Table XVII.

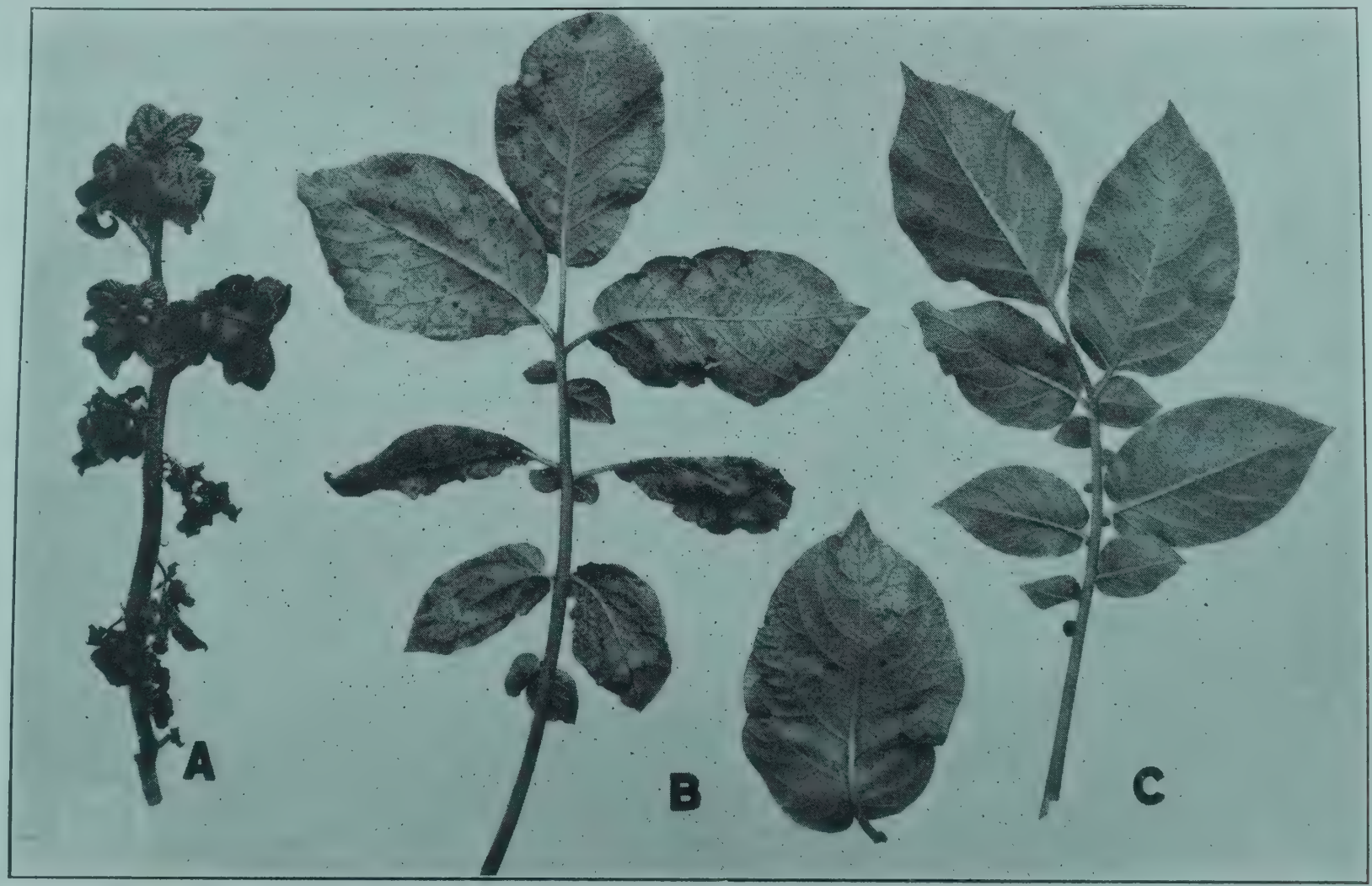
TABLE I.—*Leaf mutilation inoculations within the Green Mountain variety in the open field in 1922 and the progeny in 1923*

Inoculation series No.	Source of inoculum			Inoculated plants								Controls								
	Variety	Symptoms		1922 hills	Progeny, 1923								1922 hills	Progeny, 1923							
		1922	Progeny, 1923		Tuber units	Mild mosaic	Leaf-rolling mosaic	Rugose mosaic	Spindle tuber	Unmottled curly dwarf	Streak	Leaf-roll		Tuber units	Mild mosaic	Leaf-rolling mosaic	Rugose mosaic	Spindle tuber	Unmottled curly dwarf	Streak	Healthy
				No.	*No.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*No.*	*No.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
10	Green Mountain.	Rugose mosaic and streak.	No progeny	5	35			100			71		5	20	50					5	45
14	do	Streak	do	5	24	8		4			87		5	[a] 20	15		30				50
22	do	Unmottled curly dwarf.	Unmottled curly dwarf.	5	36					52			5	[b] 20	40	10	25				15
26	do	Spindle tuber	Spindle tuber	10	69				76				30	60				36			64
38	do	Medium mosaic	Mild, rugose, and leaf-rolling mosaic.	10	65	32		41					10	20	15		20				65
58	do	Mosaic medium+	Rugose mosaic	10	59			100					5	20	25		15		5		55
73	do	Leaf-roll	Leaf-roll	5	39								5	20							100
79	do	Rugose mosaic and spindle tuber.	Rugose mosaic and spindle tuber.	5	53			100	100				5	20							100
83	do	Rugose and leaf-rolling mosaic.	Rugose and leaf-rolling mosaic.	5	35		100	100					5	20							100
87	do	Rugose mosaic	Rugose mosaic	5	39			100					5	20							100
91	do	Water control		5	32								10	36							100
95	do	Thick-leaf mutation and spindle tuber.		5	27				63				5	16				12			88
96	do	Thick-leaf mutation.		5	29								5	20							100
97	do	Unmottled curly dwarf.	Unmottled curly dwarf.	5	25					48			5	20		5					95
101	do	Leaf-rolling mosaic.	Leaf-rolling mosaic.	5	30		33						5	20							100
122	do	Rugose mosaic and spindle tuber.	Rugose mosaic and spindle tuber.	5	28			[c]	85				5	10							100
127	do	Rugose mosaic and streak.	Rugose mosaic and streak.	5	41			68			68		5	10							100
136	do	Leaf-rolling mosaic and spindle tuber.	Leaf-rolling mosaic and spindle tuber.	10	61		100		65				10	20				20			80

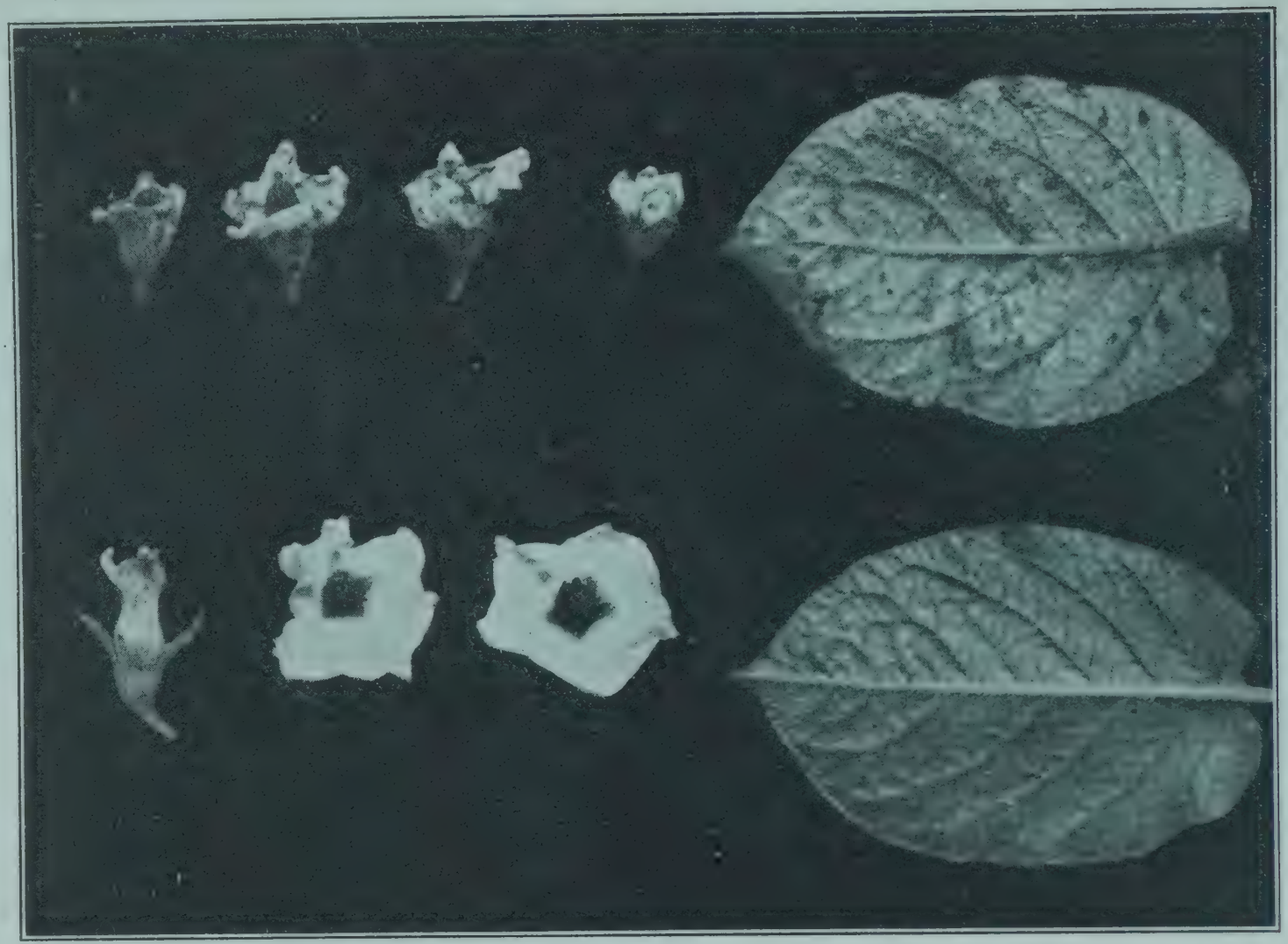
[a] 5 per cent small plants.

[b] 20 per cent small plants. Some units with more than one type of mosaic

[c] Some.



Second-year streak (A), current-season symptoms of leaf mutilation inoculation with streak in cages (B), and a healthy control leaf (C) from a sister hill to that represented by "B." Green Mountains, photographed on Sept. 6, 1923



Above from left to right, withered flower, open flowers, bud, and leaflet, showing spotting and streaking as the result of current-season leaf-mutilation inoculation with streak. Below, flowers and leaflet from an uninoculated hill of the same tuber unit as the above. Green Mountains, photographed on Aug. 4, 1922

A.—Current-season symptoms of leaf mutilation inoculation with streak in the open field. Green Mountain, photographed on Aug. 4, 1923

B and C.—Unmottled ruffled dwarf Rural New Yorker hill (B) representing the source of inoculum of series 113 of Table II, and Green Mountain hill (C) representing the progeny of the inoculated plants of this series. The Green Mountains showed a leaf-rolling-mosaic spindle-tuber combination commonly designated as curly-dwarf (distinct from unmottled curly dwarf). Photographed on Aug. 27, 1923. Compare with Plate 1, B, Plate 4, E, and Plate 5, E

D.—Unmottled curly dwarf Rural New Yorker hill representing the source of inoculum of series 111 of Table II. Photographed on Aug. 27, 1923. See Plate 5, D for the Green Mountains of this series

E.—Irish Cobbler hill representing the source of inoculum of series 139 of Table II. Photographed on Aug. 11, 1923. See Plate 5, E for the Green Mountains of this series

TABLE II.—*Intervarietal leaf mutilation inoculations of Green Mountains in the open field in 1922 and their progeny in 1923.*

Inoculation series No.	Source of inoculum: Variety	Source of inoculum: Symptoms: 1922	Source of inoculum: Symptoms: Progeny, 1923	Inoculated plants: 1922 hills	Inoculated plants: Progeny, 1923: Tuber units	Mild mosaic	Leaf-rolling mosaic	Rugose mosaic	Spindle tuber	Unmottled curly dwarf	Streak	Leaf-roll	Controls: 1922 hills	Controls: Progeny, 1923: Tuber units	Mild mosaic	Leaf-rolling mosaic	Rugose mosaic	Spindle tuber	Unmottled curly dwarf	Streak	Healthy
				No.	No.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	No.	No.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
30	Irish Cobbler	Spindle tuber	Spindle tuber	10	72				68				30	57				12			88
42	Bliss Triumph	Medium mosaic	Mild mosaic	10	67	50							5	20	20						80
46	do	Slight + mosaic	do	10	62	59							5	20	45						55
54	do	Medium + mosaic	Mild and rugose (?) mosaic	5	37	[a] 87							5	20	60						40
71	Irish Cobbler	Leaf-roll	Leaf-roll	10	59								5	19							100
111	Rural New Yorker	Curly dwarf and spindle tuber.	Curly dwarf and spindle tuber.	5	31			100		100			5	10							100
113	do	Ruffle dwarf and spindle tuber.	Ruffle dwarf and spindle tuber.	5	33		72		97				5	10	40			10			60
115	do	Dwarf; spindle tuber.	Dwarf; spindle tuber.	5	36				69				5	10							100
117	do	Ruffle dwarf in upper leaves.	Ruffle dwarf in upper leaves.	5	36		100						5	10		10					90
119	do	Mottled curly dwarf and spindle tuber.	Rugose curly dwarf.	5	31		100	100					5	10	50						50
120	do	Healthy	Healthy	5	[b] 37								5	10							100
121	do	Ruffle dwarf in upper leaves.	Ruffle dwarf	5	30		100						5	10							100
123	Seedling 39374	Rugose mosaic and spindle tuber.	Rugose mosaic and spindle tuber.	5	41			100	100				5	10				10			90
130	Seedling 41451	Streak and slight rugosity on young leaves.		5	32			100					5	10							100
133	Seedling 41491	Streaking		5	37			100					5	10							100
139	Irish Cobbler	Mild mosaic (?) and spindle tuber.	Leaf-rolling masaic and spindle tuber.	5	22		100	[c]	100				5	10							100
141	do	Leaf-rolling mosaic, rugose mosaic, and spindle tuber.	Leaf-rolling mosaic, rugose mosaic, and spindle tuber.	5	31		100	[c]	100				5	10							100
142	do	Rugose mosaic and spindle tuber.	Rugose mosaic and spindle tuber.	6	31			100	100				5	10							100

[a] 54 per cent with crinkling.

[b] Healthy.

[c] Some rugose mosaic.

modification of symptoms. These observations confirm similar phenomena recorded previously.[8] It appears that distinct ruffling of the foliage in the Rurals, even in the absence of mottling, denotes mosaic infection mainly of the leaf-rolling type (pl. 4, B and C). Curly dwarf in Rurals often indicates the presence of rugose mosaic. Curly dwarf in series 111 appeared on Green Mountains as a combination of rugose mosaic and unmottled curly dwarf. (Pls. 4, D, and 5, D.)

Streaking and slight rugosity in the seedling lot in series 130 and 133 apparently are symptoms of rugose mosaic; current-season symptoms in 1922[9] also corroborate these second generation reactions. Series 139, 141, and 142 disclose double and triple combinations of leaf-rolling and rugose mosaic and spindle tuber, with mottling often less conspicuous in Irish Cobblers than in Green Mountains (pls. 4, E and 5, E). Such combinations may become common in originally healthy Cobbler stock grown for several seasons where these various diseases are present, without mosaic being disclosed by mottling.

INTERVARIETAL LEAF MUTILATION INOCULATIONS IN INSECT CAGES

Leaf mutilation inoculations with degeneration disease combinations, between the Green Mountain and other varieties, were performed in insect cages at the same time and in the same plot as those described in Tables I and II. A major series of inoculated plants consisted of 12 plants grown from 3 tubers, each tuber having been quartered. The 4 plants from each tuber were grown respectively in 4 cages and each cage contained 3 plants, or a minor series. Thus the plants in each cage, or minor series, represented all 3 tubers of the major series, and one uninoculated minor series served as a control to the others. At the time of the first inoculation the average height of the plants was about 7 cm. One application was given to each series with the exception of series A–III and B–III which received three inoculations at weekly intervals. The results of these inoculations for 1922 and 1923 are recorded in Table III. Here each major series is denoted by a letter and each minor series or cage by the letter with a Roman numeral.

Repeated inoculations with mild mosaic in series A–III produced a higher percentage of diseased plants than the single inoculation in series E–III between the same varieties, while the same is true of spindle tuber but with a smaller difference. The symptoms in those two series appeared the same regardless of the difference in the number of inoculations. In 1923, spindle tuber appeared in every variety including the seedling. The percentage of infection with this malady was higher than with any other disease represented in these inoculations, even in the series originally healthy as indicated by the controls. No mottling resulted from inoculations with mild mosaic on Irish Cobblers in series B–III, which is in marked contrast to the high percentage of infection obtained with three applications of the same inoculum on Green Mountains in series A–III. Also, with a single inoculation, the slight amount of infection in Green Mountains, Bliss Triumphs, and seedling 39374 was absent in Irish Cobblers.

"Mosaic dwarf" inoculations produced rugose mosaic and spindle tuber in Green Mountains and in Bliss Triumphs but not in Irish Cobblers (considering the controls). In the seedling, one hill showed streak in 1922, followed in 1923 by either no growth or extreme dwarfing. These facts indicate that "mosaic dwarf" may sometimes consist of a combination of rugose mosaic, spindle tuber, and streak. The inoculum from the seedling plants produced spindle tuber in Green Moun-

[8] SCHULTZ, E. S., and FOLSOM, D. Op. cit. p. 65, 76, 79.
[9] SCHULTZ, E. S., and FOLSOM, D. Op. cit. See Table XVII.

EXPLANATORY LEGEND FOR PLATE 5

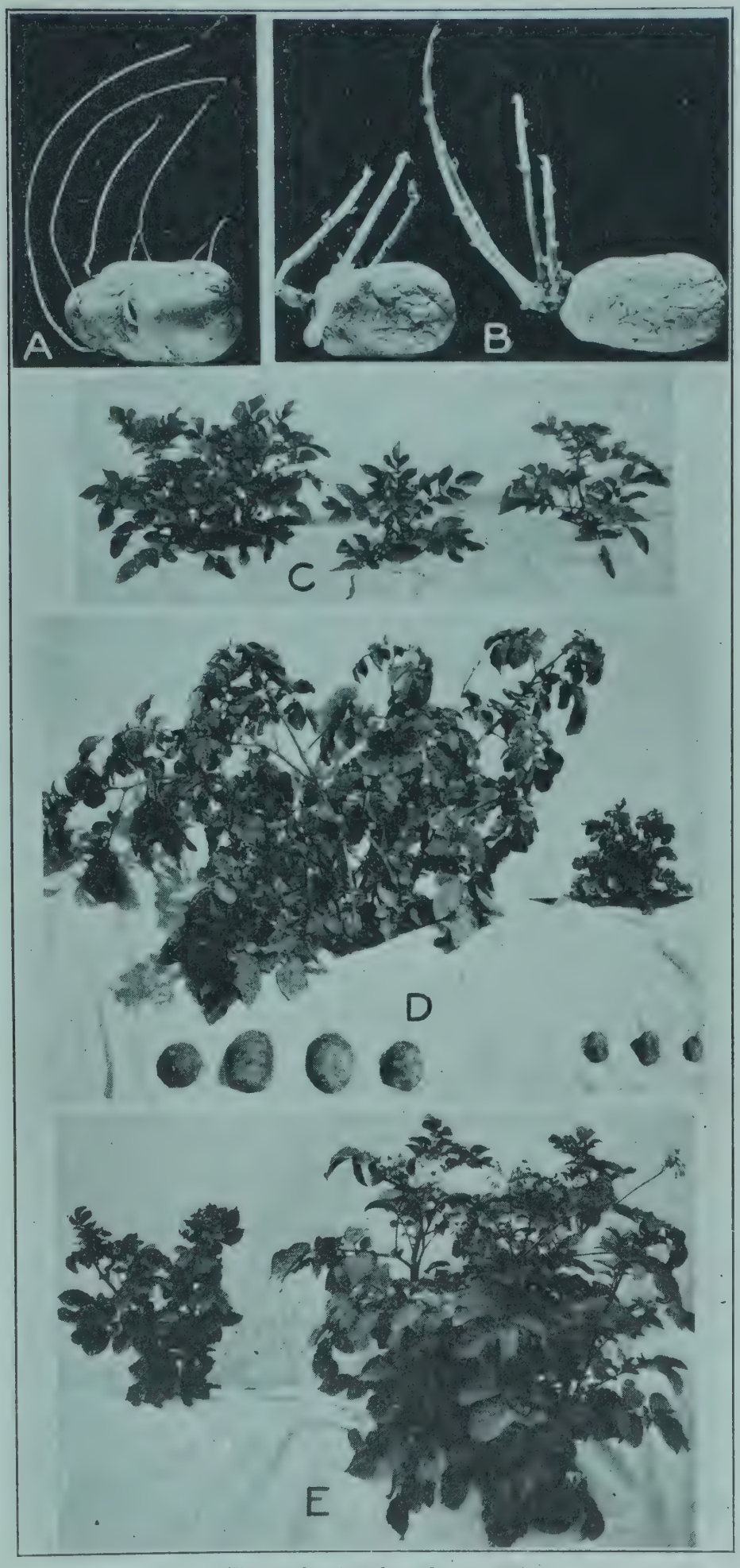
A.—Spindling-sprout tuber. Photographed on May 2, 1923. See Plate 5, B, for other tubers from the same lot, and Plate 5, C, for the plants grown from this tuber

B.—Tubers from the same lot as that shown in Plate 5, A, but without spindling sprouts. Photographed on May 2, 1923. See Plate 7, B and C for the plants grown from these tubers

C.—Plants grown from the tuber shown in Plate 5, A. Photographed on Aug. 11, 1923. Leaf-roll and mild mosaic are present

D.—Green Mountains of series 111 of Table II. The small hill represents the progeny of the inoculated hills and is infected with rugose mosaic and unmottled curly dwarf. The large hill represents the progeny of the sister hills of the inoculated hills, and is healthy. Photographed on Aug. 27, 1923. See Plate 4, D, for the source of inoculum

E.—Green Mountains of series 139 of Table II. The small hill represents the progeny of the inoculated hills and is infected with a leaf-rolling-mosaic spindle-tuber combination commonly known as curly dwarf (distinct from unmottled curly dwarf). The large hill represent the progeny of the sister hills of the inoculated hills, and is healthy. Photographed on Aug. 11, 1923. See Plate 4, E, for the source of inoculum. Compare with Plate 1, B, and Plate 4, B and C



(For explanatory legend see p 592)

TABLE III.—*Leaf mutilation inoculations in insect cages in 1922 and the progeny in 1923*

Inoculation series No.	Source of inoculum			Inoculated plants						Uninoculated controls		
	Variety	Symptoms		Variety	1922 (3 hills treated in each case)		Progeny, 1923			1922. Number of hills infected or healthy and symptoms (3 hills examined in each case)	Progeny, 1923	
		1922	Progeny, 1923		Number of hills showing symptoms	Symptoms	Tuber units		Number of tuber units infected and symptoms		Tuber units	
							Total number	Number diseased			Total number	Number infected or healthy and symptoms
A-III[a]	Green Mountain.	Mild mosaic and spindle tuber.	Mild mosaic and spindle tuber.	Green Mountain.	3	Mild mosaic; both spindling and flat oblong tubers.	14	14	13 mild mosaic; 14 spindle tuber.	3 healthy	12	12 healthy.
B-III[a]	do	do	do	Irish Cobbler		No apparent mottling; spindling tubers in hills 1 and 2, normal and spindling tubers in hill 3.	14	14	14 spindle tuber. No apparent mottling.	2 spindle tuber	18	18 spindle tuber.
E-III	do	do	do	Green Mountain.	1	Mild mosaic and spindle tuber in hill 1. Hills 2 and 3 healthy.	17	12	2 mild mosaic; 12 spindle tuber.	3 healthy	24	24 healthy.
F-III	do	do	do	Irish Cobbler	1	No apparent mottling; spindling tubers in hill 2, normal and spindling in hill 3.	11	5	5 spindle tuber. No apparent mottling.	1 spindle tuber	17	10 spindle tuber.
G-III	do	do	do	Seedling 39374		No apparent mottling.	22	21	21 spindle tuber; 2 mild mosaic.	2 spindle tuber	14	9 spindle tuber.
H-III	do	do	do	Bliss Triumph.		do	13	7	5 spindle tuber; 2 mild mosaic.	3 healthy	16	16 healthy.
E-II	do	Mosaic dwarf	Mosaic dwarf	Green Mountain.	3	Rugose mosaic	16	16	8 rugose mosaic; 13 spindle tuber.	do	24	24 healthy.
F-II	do	do	do	Irish Cobbler		Apparently healthy	13	3	3 spindle tuber	1 spindle tuber	17	10 spindle tuber.
G-II	do	do	do	Seedling 39374	1	Spotting and streaking on hill 3.	24	17	4 spindle tuber; 13 no growth.	2 spindle tuber	14	9 spindle tuber.
H-II	do	do	do	Bliss Triumph.		Apparently healthy	19	13	13 spindle tuber; 2 rugose mosaic.	3 healthy	16	16 healthy.
E-I	Seedling 39374.	Streak	Badly dwarfed and spindle tuber.	Green Mountain.	3	do	16	10	10 spindle tuber	do	24	24 healthy.
F-I	do	do	do	Irish Cobbler	3	do	16	3	3 spindle tuber	1 spindle tuber	17	10 spindle tuber.
G-I	do	do	do	Seedling 39374	3	Streak and mottling	11	11	11 badly dwarfed	2 spindle tuber	14	9 spindle tuber.
H-I	do	do	do	Bliss Triumph	3	Apparently healthy	14	1	1 spindle tuber	3 healthy	16	16 healthy.

[a] Three inoculations at weekly intervals; one inoculation in remaining series.

tains and Bliss Triumphs, nothing in Irish Cobblers, and extreme dwarfing in the seedling. In both the mosaic dwarf and streak inoculation, therefore, the seedling appeared more susceptible to streak than the other varieties.

In 1922 in the control cage of the B series, and also in that of the F series, there was a healthy hill whose 1923 progeny developed spindle tuber. Each of these hills was grown in contact with a spindle tuber hill with aphids absent. Although other evidence on transmissibility by contact, as described later, is negative in the absence of insects, it should be borne in mind that even grafting, aphid, and leaf-mutilation inoculations often give negative results. It will also be pointed out later that such diseases can spread with neither aphids nor contact serving as the apparent means. This behavior of control hills emphasizes the need of careful selection of seed tubers for cage experiments and the need of repetition and duplication of inoculation experiments. The latter need is also brought out by the facts that streak was not contracted in the mosaic dwarf and streak inoculations by Green Mountains, although spindle tuber was contracted by the same plants from the same inoculum, that streak was contracted by the seedling from the same inoculum, and that under other conditions Green Mountains have contracted streak readily from the same type of inoculation. In brief, while streak in one certain inoculum did not infect Green Mountains, it did not follow that Green Mountains were resistant to streak in other inoculations, which indicates that with such diseases one inoculation is not conclusive, especially if the results are negative.

In series A–III, E–III, and E–I, a study of the data under "Progeny, 1923," shows that the infected plants contracted either spindle tuber or both spindle tuber and mild mosaic with no explanation needed for the isolation and greater amount of spindle tuber other than the apparent greater infectiousness of spindle tuber. However, in series E–II, of the same variety as the preceding series, there was still further separation of the viruses, some plants contracting rugose mosaic, some spindle tuber, and some both. The writers have no explanation to offer for this phenomenon, in which with all conditions apparently similar, a given inoculum affects inoculated plants in two or three different ways regarding the transmission of two diseases in the inoculum. Perplexity is increased by the fact, noted above, that streak in this inoculum did not affect any plants of these Green Mountain series.

INTERVARIETAL INOCULATIONS WITH APHIDS

Inoculations of Green Mountains and Irish Cobblers with rugose mosaic, leaf-roll, and spindle tuber were conducted with aphids in 1922. Instead of dispersing from diseased to healthy plants in the same cage as in 1921,[10] the aphids were transferred from diseased to healthy plants grown in separate cages. Approximately 50 to 200 aphids per cage were introduced when the plants had reached a height of 15 to 20 cm. After feeding from 12 to 20 days the aphids were killed with nicotine sulphate spray. The results of these inoculations for 1922, as well as for the progeny in 1923, are given in Table IV.

From the data presented in Table IV it is seen that inoculation was not followed by symptoms in 1922 or in 1923 with the exception of series J, which contracted leaf-roll. Spindle tuber and mosaic in one hill of series D–I, D–II, and D–III, apparently were introduced in the open field in 1921, since the uninoculated controls in the same tuber unit show the same symptoms. In series T–II spindle tuber infection may have resulted from aphids entering the cage through a hole found in the cage late in the season. The generally negative results in this experiment disclose that under certain conditions aphids do not transmit these diseases. As noted later in Tables VII and VIII, aphids in 1923 caused infection when transferred in conditions apparently like those of the inoculation series in Table IV. Whether these interesting variations are caused by differences in temperature, light, or other factors remains to be determined by further studies on the nature of these diseases and their transmission.

CURRENT-SEASON SYMPTOMS FROM INOCULATIONS IN 1923

INTERVARIETAL LEAF MUTILATION INOCULATIONS IN THE OPEN FIELD

Leaf mutilation inoculations with different types of mosaic and with streak were made on plants of the Green Mountain variety in the open field in 1923. The effect of taking the inoculum from different parts of the

[10] SCHULTZ, E. S., and FOLSOM, D. Op. cit. See Table XVI

TABLE IV.—*Inoculations with aphids in insect cages in 1922 and the progeny in 1923*

Inoculation series No.	Source of inoculum			Inoculated plants			Uninoculated controls	
	Variety	Symptoms		Variety	Symptoms		1922 [a]	Progeny, 1923 [b]
		1922	Progeny, 1923		1922 [a]	Progeny, 1923 [b]		
C-I	Irish Cobbler	Spindle tuber	Spindle tuber	Green Mountain	3/3 healthy	20/20 healthy	3/3 healthy	24/24 healthy.
D-I	do	do	do	Irish Cobbler	1/3 spindle tuber	6/19 spindle tuber	1/3 spindle tuber	2/14 mosaic [c] and spindle tuber.
C-II	Seedling 39374	Rugose mosaic	No progeny	Green Mountain	3/3 healthy	20/20 healthy	3/3 healthy	24/24 healthy.
D-II	do	do	do	Irish Cobbler	1/3 spindle tuber	3/15 mosaic [c], 8/15 spindle tuber.	1/3 spindle tuber	2/14 mosaic [c] and spindle tuber.
C-III	Irish Cobbler	Spindle tuber	Spindle tuber and ruffled.	Green Mountain	3/3 healthy	12/12 healthy	3/3 healthy	24/24 healthy.
D-III	do	do	do	Irish Cobbler	1/3 spindle tuber	6/16 mosaic [c], 5/16 spindle tuber.	1/3 spindle tuber	2/14 mosaic [c] and spindle tuber.
T-I	Rosebush	Apparently healthy.	Apparently healthy.	Bliss Triumph	3/3 healthy	23/23 healthy		
T-II	do	do	do	do	1/3 spindle tuber	8/28 spindle tuber	6/6 healthy	6/6 healthy.
I	Irish Cobbler	Leaf-roll	Leaf-roll	Green Mountain	2/2 healthy	16/16 healthy		
J	do	do	do	Hebron	2/2 healthy	6/14 leaf-roll		
K	do	do	do	do	3/3 healthy	21/21 healthy		

[a] The two numbers in each fraction denote, respectively, the number of hills infected (or healthy) and the total number.
[b] The two numbers in each fraction denote, respectively, the number of tuber units infected (or healthy) and the total number.
[c] Mosaic in this variety was not marked, and was present in 1923 probably because of the generally favorable seasonal conditions for mosaic.

plant was tested with rugose mosaic in series 5 to 9. The remaining series represent inoculations comparable with similar series conducted in cages, the results of which will be noted later. The current-season effects of these inoculations are represented in Table V.

From the data on series 5 to 9 it is apparent that inoculum from either the entire shoots, the stems and petioles, or the leaflets alone, was more effective than that from roots or tubers; in fact, the very low percentage of infection that followed inoculations from roots or tubers may perhaps be due to field infection, although the uninoculated controls in these series remained healthy. The practically negative results from the inoculation with juice from the seed tubers is somewhat surprising, considering the facts that they perpetuate the disease and that grafting causes infection. These seed tubers were unplanted ones from the same lot as those from which the plants were grown that served as the source of inoculum for series 5 to 8; the whole lot came from diseased stock and all that were planted produced diseased plants. Inoculation with juice from the roots also gave practically negative results, although they were taken from the same plants as the shoots, leaflets, and stems and petioles, showing that not all parts of a plant contain the virus in a similar state of infectiousness. Infectiousness here seems to be correlated with the presence of chlorophyll. Not only were the colorless parts far less infective, but the inoculum from the leaflets was more infectious than that from the supporting parts of the plant, the latter infecting only 75 per cent of the plants inoculated instead of all of them. Also the inoculum from the leaflets was more infectious than that from the leaflets, petioles, and stems (aerial parts) combined, because, while infecting no more plants, still it infected them more completely by August 14, as is shown by Figure 1. As this figure indicates, of the 20 plants infected from the leaflets (in section W–7, row 2), 16 were obviously diseased in the upper leaves of all the shoots by August 14, while of the 20 plants infected from the shoots (in row 4), only 9 were thus affected. The inoculum was used from an uncovered dish; possibly when present chlorophyll prevented a sterilization of the inoculum by light.

Inoculations with mild mosaic (in series 14 of Table V) induced the appearance of no current-season symp-

TABLE V.—*Leaf mutilation inoculations of Green Mountains in the open field in 1923*

Inoculation series No.	Source of inoculum			Inoculated plants			Uninoculated controls	
	Variety	Symptoms		Total number	Number infected	Symptoms	Number of hills	Symptoms
		1922	Progeny, 1923					
5	Green Mountain.	Rugose mosaic.	Rugose mosaic, roots.	10	1	Rugose mosaic	30	Healthy.
6	do	do	Rugose mosaic, leaflets.	20	20	do	20	Do.
7	do	do	Rugose mosaic, stems and petioles.	20	15	do	20	18 hills healthy.
8	do	do	Rugose mosaic, shoots.	20	20	do	20	Healthy.
9	do	do	Seed tubers	16	2	do	24	Do.
10	do	Healthy	Healthy	17	0	Healthy	19	Do.
14	do	Mild mosaic.	Mild mosaic	10	0	Apparently healthy	10	Do.
16	do	Leaf-rolling mosaic.	Leaf-rolling mosaic.	10	10	Leaf-rolling mosaic	10	Do.
18	do	Rugose mosaic.	Rugose mosaic	5	5	Rugose mosaic	15	Do.
20	do	Streak	Streak and slight mottling.	5	1	Streak	15	Do.
22	do	Healthy	Healthy	10	0	Healthy	30	Do.
29	Irish Cobbler.		Mild mosaic (?)	10	0	do	30	Do.
30	do		Rugose mosaic with streaking.	5	5	Rugose mosaic including streaking, spotting and burning.	15	Do.
31	do		do	10	8	Rugose mosaic in 6 hills. Rugose mosaic including streaking, spotting and burning in 2 hills.	30	Do.

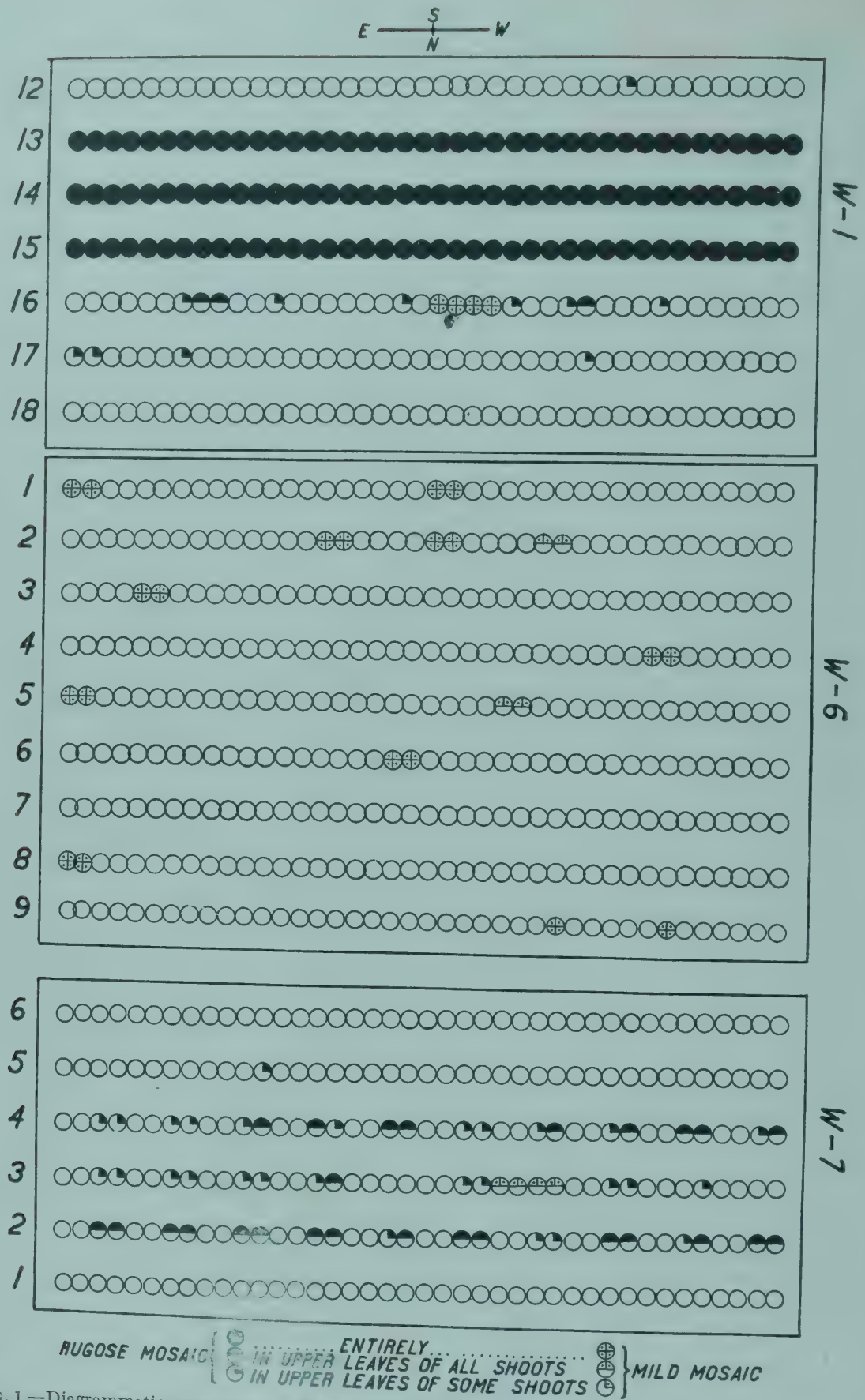


FIG. 1.—Diagrammatic representation of the results of inoculation with rugose mosaic and of proximity in 1923 to plants with this disease. Each circle represents a hill (drawn to scale as of one foot in diameter of each circle), with disease indications as explained in the legend. See also Plate 9, A. In Section W-1, planted on May 15, and observed, July 31, row 12 was planted with control healthy stock, rows 13 to 15 with seed from rugose mosaic plants, and rows 16 to 18 as for row 12. Section W-6 was planted on May 19, as a control plot, from the same stock as the control rows in Section W-1, and was observed on Aug. 14. Section W-7 was planted on June 19 and 22 with the same control stock as above, was inoculated on July 14, and was observed on August 14. In this section, hills of row 1 were inoculated with juice from the roots of rugose mosaic plants, row 2 from the leaflets, and row 3 from the stems and petioles, row 4 from the shoots (stems and leaves), row 5 from the seed tubers, and row 6 with tap water. On this section each row consists of 10 four-hill tuber units, and one or two hills in each tuber unit were inoculated, these being the two right-hand or west hills in each tuber unit in rows 2, 3, and 4. In section W-6, each row consists of 20 two-hill tuber units. For further discussion see the text on pp. 507 and 509

toms, which is in marked contrast to the complete positive results obtained with leaf-rolling and rugose mosaic in the open (series 8, 16, and 18) and with mild mosaic in cages (described later). In series 29, a mild type of mottling, tentatively called a mosaic, on Irish Cobblers likewise failed to produce current-season symptoms on inoculated Green Mountains. Negative results with a similar type of so-called mosaic on Irish Cobblers were obtained with stalk grafts on 12 Green Mountain shoots in three separate tuber units in cages. This seems to suggest that the mottling on the Irish Cobblers is not infectious, but to verify this conclusion similar negative reactions in the second generation are essential. In any case this mottling on Irish Cobblers appears distinct from rugose mosaic, as described previously and as disclosed by the positive current-season reactions in series 30 and 31; in these series almost 100 per cent infection resulted from rugose mosaic inoculations performed under the same conditions as for the mottled Irish Cobbler inoculation.

In connection with the use of juice from seed tubers as described above, the results of tests of knife transmission may be noted. The use of the same knife to cut alternately diseased and healthy tubers at planting time, with 10 healthy tubers thus inoculated with each disease, did not result in transmission. Each of the four types of mosaic, spindle tuber, unmottled curly dwarf, leaf-roll, and several combinations were tested.

INTERVARIETAL LEAF MUTILATION INOCULATIONS IN INSECT CAGES

In order to determine the comparative reactions of the Green Mountain, Irish Cobbler, and Rural New Yorker varieties to mild, leaf-rolling, and rugose mosaic, and of Green Mountains to streak, leaf mutilation inoculations were performed in insect cages in 1923. Inoculations with mosaic were made on the same date, when the height of the plants varied from 7 to 30 cm. Diseased Green Mountains served as the sources of inoculum. The method of planting three hills in each cage, from three tuber units, respectively, resembled that previously described for cage experiments. The current-season results of these inoculations are recorded in Table VI.

From the results presented in Table VI it is quite apparent that rugose mosaic in series A–3, B–3, and C–3 produced complete infection in the three varieties. Inoculations with mild and leaf-rolling mosaic were equally effective on Green Mountains (pl. 6, A) but not apparently so on Irish Cobblers and Rural New Yorkers.[11] It is also noteworthy that the first symptoms of mild mosaic, leaf-rolling mosaic, and rugose mosaic appeared, respectively, 32, 25, and 18 days after inoculation, indicating different incubation periods for these mosaic types. The absence of current-season symptoms with mild mosaic on Irish Cobblers and Rural New Yorkers suggests either differences in varietal susceptibility or varietal modification of symptoms of this disease. Reactions resulting from aphid inoculations have been recorded.[12] Table VII in this paper gives the data from later work. Streak was transmitted to Green Mountains resulting in the characteristic current-season symptoms (pl. 2, B).

INTERVARIETAL INOCULATIONS WITH APHIDS

For comparison with the leaf-mutilation inoculations described in the preceding section of this paper, inoculations were performed with aphids in insect cages in the field in 1923. Aphids, *Macrosiphum solanifolii* Ashm., obtained from rose bushes, were transferred to healthy Bliss Triumphs and then to Green Mountains affected respectively with different types of mosaic. After feeding for about 10 days on diseased plants, approximately 50 to 100 aphids per hill were introduced to healthy caged plants varying in height from 7 to 30 cm. After feeding from 12 to 13 days on the inoculated plants the aphids were killed with nicotine sulphate spray. Current-season reactions are recorded in Table VII.

As is shown in Table VII, of the three varieties, Green Mountain, Irish Cobbler, and Rural New Yorker, that were tested, only the Green Mountain showed positive results with mild and leaf-rolling mosaic. All three varieties showed slight symptoms resulting from inoculations with rugose mosaic. However, the reactions to rugose mosaic were not so pronounced as those with the leaf mutilation inoculations denoted in Table VI. No current-season symp-

[11] Since the submittal of this manuscript observations on the second generation disclosed mild mosaic on Rural New Yorker and leaf-rolling mosaic on Irish Cobbler and Rural New Yorker.

[12] SCHULTZ, E. S., and Folsom, D. Op. cit. p. 65–84.

TABLE VI.—*Leaf mutilation inoculations in insect cages in 1923*

Inoculation series No.	Symptoms in source of inoculum [a]		Inoculated plants (3 hills in each case)					Uninoculated controls	
	1922	Progeny, 1923	Variety	Height when inoculated	Date of inoculation	Date of first symptoms	Number of hills infected and symptoms	Number of hills	Number of hills infected and symptoms
				Cm.					
A-1	Mild mosaic	Mild mosaic	Green Mountain	15–25	July 16	Aug. 17	3 mild mosaic	3	3 healthy.
B-1	do	do	Irish Cobbler	17	do		3 apparently healthy	3	Do.
C-1	do	do	Rural New Yorker	15–25	do		do	3	Do.
A-2	Leaf-rolling mosaic	Leaf-rolling mosaic	Green Mountain	20		Aug. 10	3 leaf-rolling mosaic	3	Do.
B-2	do	do	Irish Cobbler	10–15	July 16	Aug. 15	1 slight mottling on axillary shoots	3	Do.
C-2	do	do	Rural New Yorker	7–30	do		3 apparently healthy	3	Do.
A-3	Rugose mosaic	Rugose mosaic	Green Mountain	17	do	Aug. 3	3 rugose mosaic	3	Do.
B-3	do	do	Irish Cobbler	15	do	Aug. 15	do	3	Do.
C-3	do	do	Rural New Yorker	7	do	do	do	3	Do.
G-9	Streak	Streak and slight mottling	Green Mountain	15–17	July 17		3 apparently healthy	3	Do.
G-10	do	do	do	15–17	do	Aug. 15	2 streak without mottling	3	Do.

[a] All sources were plants of the Green Mountain variety.

TABLE VII.—*Inoculations with aphids in 1923*

Inoculation series No.	Symptons in source of inoculum [a]		Inoculated plants (3 hills in each case)					Uninoculated controls	
	1922	Progeny, 1923	Variety	Height when inoculated	Aphids: Approximate number per hill	Aphids: Feeding period	Number of hills infected and symptoms	Hills	Symptoms
				Cm.		*Days*			
A–5	Mild mosaic	Mild mosaic	Green Mountain.	17	100+	13	2 mild mosaic	3	Healthy.
B–5	do	do	Irish Cobbler	15	100±	13	3 apparently healthy	3	Do.
C–5	do	do	Rural New Yorker.	7–15	100±	13	do	3	Do.
A–6	Leaf-rolling mosaic	Leaf-rolling mosaic	Green Mountain.	17–30	50±	13	3 leaf-rolling mosaic	3	Do.
B–6	do	do	Irish Cobbler	15–25	50±	12	3 apparently healthy	3	Do.
C–6	do	do	Rural New Yorker.	7–15	50±	12	do	3	Do.
A–7	Rugose mosaic	Rugose mosaic	Green Mountain.	20–25	50±	12	1 slight rugose mosaic	3	Do.
B–7	do	do	Irish Cobbler	17	50±	12	1 slight mottling on young shoots	3	Do.
C–7	do	do	Rural New Yorker.	10–15	50±	12	1 streaking and rugosity of foliage	3	Do.
G–7	Streak	Streak and slight mottling	Green Mountain.	16	50+	13	3 apparently healthy	3	Do.
D–1	Unmottled curly dwarf	Unmottled curly dwarf	do	17	50+	13	3 one tuber per hill somewhat spindling.	3	Do.
E–1	do	do	Irish Cobbler	17	50+	13	2 one tuber per hill spindling	3	Do.
F–1	do	do	Rural New Yorker.	10–15	50+	13	1 one tuber somewhat spindling	3	Do.

[a] All sources were plants of the Green Mountain variety.

toms appeared on the foliage of plants inoculated with unmottled curly dwarf in series D–1, E–1, and F–1; however, some tubers in this series were more or less spindling. The negative results obtained with aphid inoculations of Irish Cobblers and Rural New Yorkers with mild mosaic apparently suggest either varietal modification of symptoms or differences in susceptibility to this disease. Similar reactions were described in the preceding section of this paper. The streak inoculation with aphids was not followed by symptoms as with leaf-mutilation inoculation (see Table VI).[13]

Some comment may appropriately be made here upon the still frequently repeated statement that mosaic becomes worse as the number of years increases since it has infected a given stock. The statement in question does not refer so much to the often demonstrated fact that the percentage of mosaic may increase in a stock until the latter is entirely diseased, but rather implies that mosaic is mild at first, then medium, and finally severe. According to this theory it is hard to explain the results described in this and the previous sections of the present paper, where inoculum introduced by means of leaf mutilation or aphids did not invariably produce mild mosaic as the first-year symptoms, but instead produced respectively the same symptoms as were observed on the plants from which the inoculum was obtained (pl. 6, A and B). It seems preferable to maintain that there are several types of mosaic, each retaining its characteristic symptoms indefinitely under the same environmental and varietal conditions, while being perpetuated by the tubers or even when transmitted from one plant to another.

In this connection it may be added that the mild mosaic plants from which the first Green Mountain series in Table VI and in Table VII were infected, were known to be of the eighth generation of a strain originating from mild mosaic hills selected in 1916. During that time the stage of mosaic had not become worse (pl. 7, A), although of course there was some variation with the seasons and some other progeny of the original selections had acquired additional diseases that made them appear to have curly dwarf, bad mosaic, and mosaic dwarf.

It may also be suggested that in applying these methods to potato degeneration problems in other regions, it will be found helpful to use insect cages with openings in the top (pl. 8, A) this feature facilitating the proper care of a large number of cages (pl. 8, B).

INTERVARIETAL DISEASE-COMBINATION INOCULATIONS WITH APHIDS

Combination inoculations with mild, leaf-rolling, rugose, and crinkle mosaic, leaf-roll, and spindle tuber, were made on Green Mountains, Irish Cobblers, and Rural New Yorkers in insect cages in the field. Aphids were introduced into each cage from plants obtained from two sources and with respectively different diseases. The methods of inoculation, including the aphid transfers, resembled those described in the previous section of this paper. In each series aphid transfers were made from the two sources on the same date with the exception of series G–2, G–3, and G–5, in which aphids from leaf-rolling mosaic plants were introduced to healthy plants 13, 7, and 12 days, respectively, before the aphids from the other disease in the combination were transferred.

Approximately 50 to 100 aphids were introduced to each of the three hills in a cage when the height of the plants varied from 7 to 40 cm. The current-season results of these inoculations are indicated in Table VIII.

The data presented in Table VIII show that combinations of rugose mosaic and spindle tuber became apparent in series D–3 and E–3; of crinkle mosaic and spindle tuber in series G–1; and of leaf-rolling mosaic and leaf-roll in series G–3.

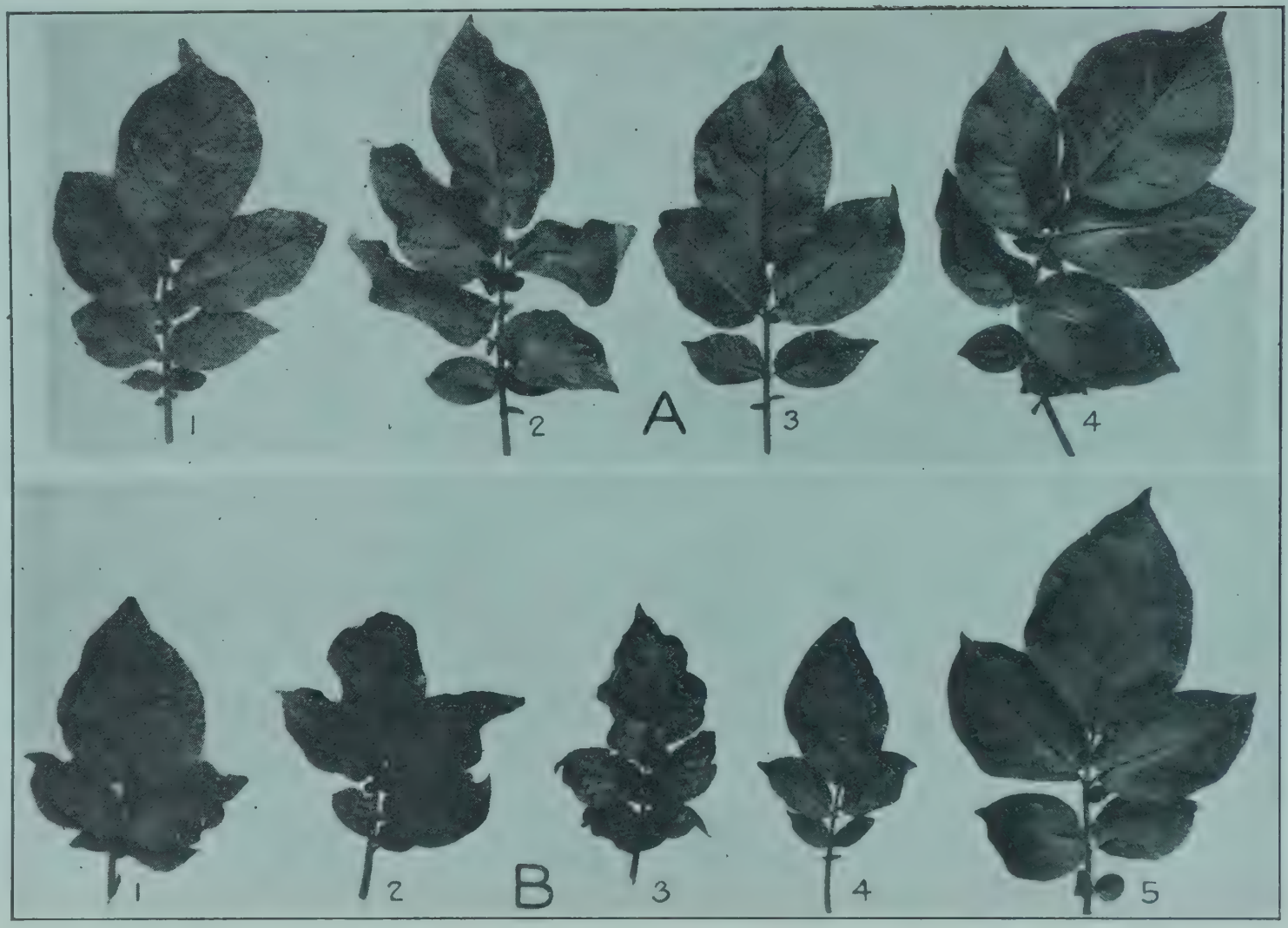
It is noteworthy that leaf-roll in the leaf-rolling mosaic and leaf-roll combination retained the characteristic symptoms of leaf-roll with leaf-rolling

[13] Since the submittal of this manuscript, observation on the second-generation plants disclosed typical second-generation streak symptoms. Aphid transmission of streak has also been reported by Atanasoff (p. 32, Rpt. Internat. Confer. Phytopath. Econ. Entom. Holland, 1923).

EXPLANATORY LEGEND FOR PLATE 6

A.—Representative leaves from 4 hills of the same Green Mountain tuber unit grown in cages of Table VI and infected with the leaf-mutilation method as follows: 1, cage A–3, rugose mosaic; 2, cage A–2, leaf-rolling mosaic; 3, cage A–1, mild mosaic; 4, cage A–4 (control to others), healthy. Photographed on Sept. 6, 1923. Compare with Plate 6, B

B.—Representative leaves from 5 Green Mountain hills inoculated in insect cages with aphids and infected with rugose mosaic (1), leaf-rolling mosaic (2), crinkle mosaic (3), and mild mosaic (4), and healthy (5). Photographed on Sept. 6, 1923. Compare with Plate 6, A



(For explanatory legend see p. 512.)

mosaic symptoms added, demonstrating a distinction between these two diseases. Similar distinctions appeared in the other series. Usually more marked dwarfing resulted from these combinations than from the single diseases.

CONTACT INOCULATIONS IN THE GREENHOUSE

In order to ascertain whether the mere contact of the seed pieces, roots, and shoots of spindle-tuber plants with those of healthy plants would produce infection, seed pieces from diseased and healthy plants were planted together in 10-inch pots in the Washington greenhouses during the winter of 1922–23. The healthy plants were of three varieties, Green Mountain, Irish Cobbler, and Bliss Triumph. Each variety was represented by 14 healthy tubers which were split in two. One-half of each healthy Green Mountain and Irish Cobbler tuber was planted in contact with a spindle tuber Bliss Triumph seed piece, while a half of each healthy Bliss Triumph tuber was planted in contact with a spindle tuber Green Mountain seed piece. Here contact of freshly cut surfaces was not intimate, as in tuber grafting. The other healthy tuber halves served as controls and were planted in 8-inch pots. From the time of planting, January 5, 1923, until harvest, April 12, 1923, the forty-two 10-inch pots were covered with aphid-proof cages, while the control plants in 8-inch pots remained uncaged in the same greenhouse.

Three observations during this experiment did not disclose any insects on the caged plants. On account of seed piece decay six of the healthy Green Mountains in 10-inch pots failed to produce tuber progeny.

At harvest all tuber progeny from the contact healthy plants as well as from the control plants appeared normal, while the tuber progeny from the spindle tuber plants which had attained sufficient size for diagnosis were spindle-shaped.

The second generation Irish Cobbler and Bliss Triumph plants were grown in the open field at Presque Isle, Me., and those of the Green Mountain variety in the Washington, D. C., greenhouse. In this generation 31 Irish Cobbler tuber units, representing 13 of the original contact half tubers (one half tuber being without tuber progeny), as well as the respective controls, were healthy. Spindle tuber progeny resulted from all the original spindle tuber plants. In the second generation 29 Bliss Triumph tuber units, representing 11 contact half tubers of the first generation and their respective controls, remained free from spindle tuber. One other contact half tuber and its respective control produced spindle tuber plants (in the first generation in the greenhouse) possibly as a result of late season field infection the previous season. Two contact half tubers failed to produce tubers in the first generation because of seed-piece decay. Likewise, in the second generation 22 Green Mountain tuber units, representing 11 half tubers of the first generation and the respective controls, failed to produce spindle tuber progeny.

These observations confirm similar ones on the contact of mosaic and leaf-roll plants with insects excluded, where negative evidence was obtained.[14]

A similar experiment but without insect cages, was performed in the Orono, Me., greenhouse at about the same time. Here Bliss Triumph tubers that were healthy or with mosaic of several types, leaf-roll, or spindle tuber were planted in steam-sterilized soil, each in a 10-inch pot with a healthy Green Mountan tuber. There were two similar series, W and C, which were placed in separate rooms, the former in a warmer place than the latter. Planting was done in the second week of December, 1922. White flies (*Asterochiton vaporariorum*) appeared in January on other plants in the same greenhouse and were cyanide fumigated on January 12 and 18. All but one of the Bliss Triumph plants had emerged from the soil by January 22, and the last one and also the Green Mountains had emerged by February 8. White flies were present again, on the C series this time, in February and later in April, and were cyanided each time. Frequent observations disclosed no aphids at any time, in this

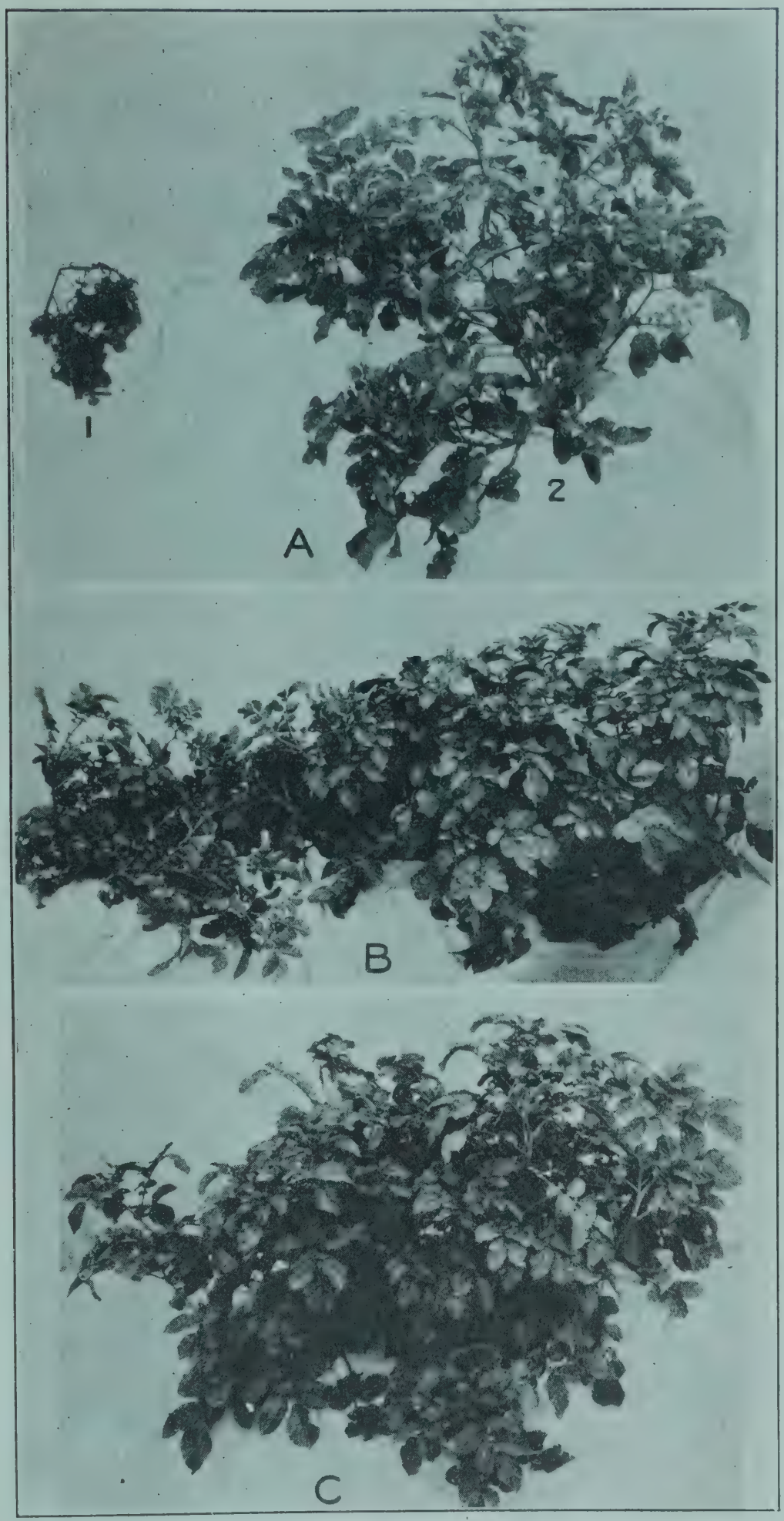
[14] SCHULTZ, E. S., and FOLSOM, D. Op. cit. p. 46–48, 54.

EXPLANATORY LEGEND FOR PLATE 7

A.—Mild mosaic (2) in a Green Mountain hill of a strain known to be entirely mosaic from 1916 to 1923, inclusive, compared with a rugose mosaic hill of the same variety (1). Photographed on Sept. 10, 1923, after the death of the rugose mosaic vine

B.—Plants grown from the right-hand tuber of Plate 5, B. Photographed on Aug. 11, 1923. Mi mosaic is present. Compare with Plate 5, C

C.—Plants grown from the left-hand tuber of Plate 5, B. Otherwise as for Plate 7, B



(For explanatory legend see p. 514)

TABLE VIII.—*Disease combination inoculations with aphids in 1923*

Inoculation series No.	Symptoms in sources of inoculum [1]		Inoculated plants (3 hills in each case)					Controls	
	1922	Progeny, 1923	Variety	Height when inoculated	Aphids: Approximate number per hill	Aphids: Feeding period	Number of hills infected and symptoms	Hills	Symptoms
				Cm.		*Days*			
D-2	Mild mosaic Spindle tuber	Mild mosaic Spindle tuber	Green Mountain	15 15	50+ 50+	13 13	2 one tuber per hill somewhat spindling	3	Healthy.
D-3	Rugose mosaic Spindle tuber	Rugose mosaic Spindle tuber	do	15–20 15–20	50± 50±	13 13	3 rugose mosaic with streaking in 1 hill	3	Do.
E-2	Mild mosaic Spindle tuber	Mild mosaic Spindle tuber	Irish Cobbler	15 12–17	50+ 50±	14 13	2 one tuber per hill somewhat pear shaped 3 one tuber per hill somewhat spindling	3 3	Do.
E-3	Rugose mosaic Spindle tuber	Rugose mosaic Spindle tuber	do	12–17 12–17	50± 50±	13 13	2 rugose mosaic and 1 tuber per hill somewhat spindling. Third hill with two tubers somewhat spindling.	3	Do.
F-2	Mild mosaic Spindle tuber	Mild mosaic Spindle tuber	Rural New Yorker	7–15 7–15	50+ 50±	14 13	1 three tubers spindling	3	Do.
F-3	Rugose mosaic Spindle tuber	Rugose moasic Spindle tuber	do	15–20 15–20	50± 50±	13 13	One tuber in 1 hill and 2 tubers in another hill spindling	3	Do.
G-1	Crinkle mosaic [2] Spindle tuber	Crinkle mosaic [2] Spindle tuber	Green Mountain	15 15	50+ 50+	13 13	3 crinkle mosaic. Two tubers in hill 1 and one tuber in hill 2 somewhat spindling	3	Do.
G-2	do Leaf-rolling mosaic	do Leaf-rolling mosaic	do	40 13–20	50+ 50+	13 13	3 leaf-rolling mosaic	3	Do.
G-3	do Leaf-roll	do Leaf-roll	do	13–15 30–35	50+ 100+	13 13	3 leaf-rolling mosaic and leaf-roll	3 3	Do. Do.
G-5	Rugose mosaic Leaf-rollIng mosaic	Rugose mosaic Leaf-rolling mosaic	do	30–35 15–20	100+ 50+	13 17	1 leaf-rolling mosaic and possibly rugose mosaic	3	Do.
G-6	Mild mosaic Streak	Mild mosaic Streak	do	17–35 17–35	50+ 50+	13 13	1 mild mosaic	3	Do.

[1] All of the Green Mountain variety.

[2] Apparently a fourth type of mosaic; probably not identical with the "crinkle" of other writers.

respect duplicating conditions of the preceding winter in this greenhouse. Thrips were present as usual, being more abundant where the W series was grown. The plants in these series were observed frequently and the tubers when dug, on April 4, were also examined. Conditions for spindle tuber diagnosis were considered somewhat unfavorable. The tubers were kept in cold storage during the summer. The plants of the second generation were grown in the latter months of 1923, one only from each tuber. The mosaic and leaf-roll observed in the two generations designated, respectively, because of the time of planting as those of 1922 and 1923, although both were grown in 1923, are given in Table IX.

It will be seen from this table that about two-thirds of the Bliss Triumphs were mosaic, with one healthy 1922 hill producing mosaic progeny. Of the Green Mountains, about two-thirds were grown in 1922 in root contact with Bliss Triumph mosaic hills, that is, 24 of a total of 37. Of the 24, 5 produced mosaic progeny; and of the 37, 7 produced mosaic progeny. The difference of 2 represents two plants in root-contact with leaf-roll Bliss Triumphs. None of the Green Mountains contracted leaf-roll. Evidently conditions were favorable for the spread of mosaic both with and without root contact, but not for the spread of leaf-roll. The abundance of mosaic plants and the arrangement of the plants made leaf contact with mosaic plants possible for the two Green Mountain hills that were not in root contact and that became mosaic. It will be noted that only one tuber (of only one hill, of course) became mosaic in the C series, where white flies were present. The greater number of infections occurring in the W series leads to the suggestion of the possibility of transmission by thrips;[15] this difference, together with the fact that some of the 1922 hills had both diseased and healthy progeny, also indicates that the infection of the Green Mountains occurred in the greenhouse and was not present in the tubers when they were planted in 1922. Inasmuch as thrips can be controlled readily in experiments and have no practical importance as to potatoes, no tests are planned to test this suggestion about their transmitting potato mosaic. It remains clear, however, that mosaic can spread in the absence both of aphids and of artificial inoculation.

TABLE IX.—*Effect of proximity to disease in the absence of aphids*

	Number of hills, 1922			Number of hill lots, 1923 [a]			Number of hills, 1923 [b]		
	Total	Mosaic	Leaf-roll	Total	Mosaic	Leaf-roll	Total	Mosaic	Leaf-roll
W—Green Mountain series:									
In root contact with Bliss Triumphs in 1922	20	0	0	20	[c] 6	0	73	18	0
In root contact with mosaic Bliss Triumphs 1922	14	0	0	14	[c] 5	0	51	14	0
In root contact with leaf-roll Bliss Triumphs [d] in 1922	2	0	0	2	1	0	8	4	0
W—Bliss Triumph series	20	14	[d] 2	20	14	2	100	69	8
C—Green Mountain series:									
In root contact with Bliss Triumphs in 1922	17	0	0	17	[c] 1	0	65	1	0
In root contact with mosaic Bliss Triumphs in 1922	10	0	0	10	0	0	41	0	0
In root contact with leaf-roll Bliss Triumphs in 1922	2	0	0	2	[c] 1	0	9	1	0
C—Bliss Triumph series	17	10	[d] 2	17	11	2	85	54	4
W and C—Green Mountain series:									
In root contact with Bliss Triumphs in 1922	37	0	0	37	[c] 7	0	138	19	0
In root contact with mosaic Bliss Triumphs in 1922	24	0	0	24	[c] 5	0	92	14	0
In root contact with leaf-roll Bliss Triumphs in 1922	4	0	0	4	[c] 2	0	17	5	0
W and C—Bliss Triumph series	37	24	[d] 4	37	25	4	185	123	12

[a] Each from a hill of 1922.
[b] Each hill grown from a different tuber (produced in 1922).
[c] Including those partly diseased.
[d] None of the leaf-roll Bliss Triumphs were also mosaic.
[e] Partly diseased.

[15] Thrips are suspected as a carrier of a mosaic of Eucharis lilies. WHETZEL, H. H. REPORT OF THE PLANT PATHOLOGIST FOR THE PERIOD JANUARY 1ST TO MAY 31ST, 1922. Bermuda Rpts. Bd. and Dept. Agr. 1922: 29, 30–31, 1923.

INOCULATIONS WITH APHIS ABBREVIATA

Three genera of aphids have been found on potatoes in northeastern Maine, represented by *Macrosiphum solanifolii* Ashmead, *Myzus persicae* Sulz., and *Aphis abbreviata* Patch.[16] It is well known from previous studies by the writers and others that *Macrosiphum solanifolii* and *Myzus persicae* transmit virus diseases of the potato and other cultivated plants.[17] *Aphis abbreviata* feeds very generally on the lower leaves of the potato. By color, size, and shape it can be distinguished readily from the other species when on potatoes. Since *Aphis abbreviata* has been observed on potato plants barely 12 cm. high, it is apparent that it may sometimes be present before it is possible to eliminate diseased plants even in the first roguing.

During 1923 when *Aphis abbreviata* appeared to be especially numerous in comparison with previous years and in comparison with the other species, inoculation experiments with this species were begun. Since the overwintering host of *Aphis abbreviata* was not yet definitely known, colonies from potato foliage were used for inoculations. This procedure resulted unfortunately in the introduction of a few individuals of *Macrosiphum solanifolii*, so that the positive results with *Aphis abbreviata* in field cages in the first test were not conclusive.

However, later in the season pure *Aphis abbreviata* colonies were secured from E. M. Patch, as well as from isolation cultures in field insect cages. After these aphids colonized on mild mosaic caged Green Mountain plants for a few weeks, about 300 of them were transferred to each of three caged healthy Green Mountain hills when the plants were about 30 cm. high. At the same time transfers from the same colony were made to sprouts of three healthy Green Mountain half tubers. The lateness of the season apparently prevented sufficient additional foliage growth for the appearance of current-season symptoms in the cages. However, in the greenhouse second generation plants from a tuber from each of the three caged hills showed mild mosaic throughout the plants. The control plants from the three sister tuber progeny were healthy. Of the plants grown from three sprouted half tubers inoculated with *Aphis abbreviata* from mild mosaic Green Mountain plants, two developed mild mosaic when about 20 cm. high, while the corresponding half-tuber controls, from the same tubers as the inoculated plants, remained healthy.

NATURAL SPREAD OF MOSAIC WITH CURRENT-SEASON SYMPTOMS

In 1923 colonies of apterous *Aphis abbreviata* appeared somewhat earlier and in greater numbers than those of the other species on the disease-propagating plot, the first colonies being observed when the potato plants were scarcely 12 cm. above ground. Following this, current-season rugose mosaic symptoms (pl. 9, A) appeared in healthy stock in rows near or adjacent to rows of diseased plants. This is shown diagrammatically in Figure 1. In section W–1, 60 per cent of the four-hill tuber units and 23 per cent of the hills in the first row (16) on the north of three rugose mosaic rows (13–15) were rugose mosaic in the upper leaves of part or of all of the shoots by July 31. The disease also spread to the first row on the south and to the second row on the north with similar current-season effects but to a less extent. The difference between the two adjoining rows on the north and south indicates transmission by insects rather than by root contact.

[16] PATCH, E. M. THE BUCKTHORN APHID. Me. Agr. Exp. Sta. Bul. 317: 29–52, illus. 1924.
[17] SCHULTZ, E. S., and FOLSOM, D. Op. cit.

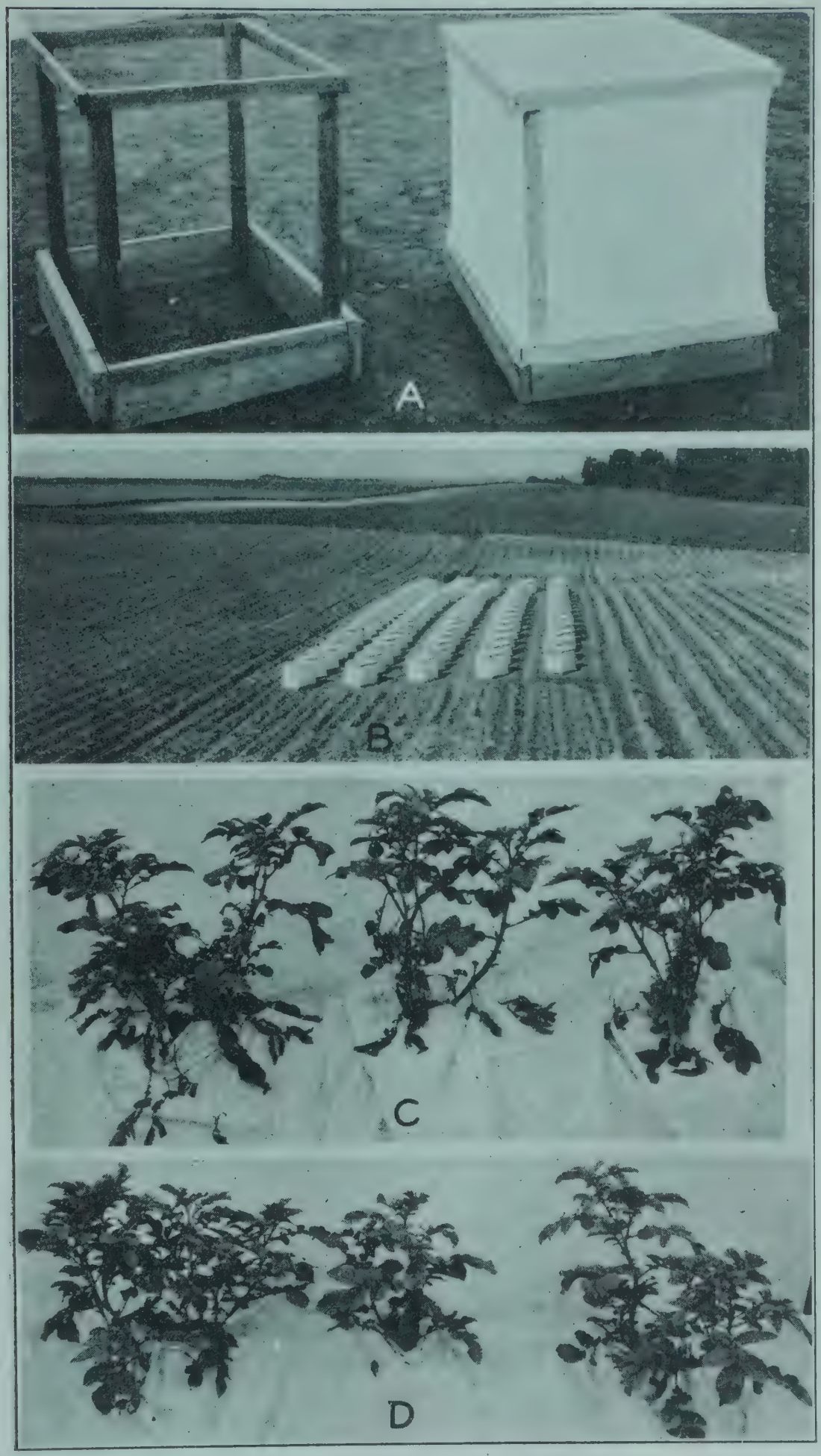
EXPLANATORY LEGEND FOR PLATE 8

A.—Frame and completed insect cage suitable for 3 potato hills. The cloth is in two pieces, one covering the four sides and the other across the top with an overlap in the middle and a slit in the center cut at right angles to the overlap

B.—Insect cages used in 1923, each covering 3 potato hills. The top opening (A) facilitates the proper care of a large number of cages. The arrangement shown permits the inclusion of the maximum number of hills possible with the available light

C.—Green Mountain tuber unit showing delayed symptoms of rugose mosaic after perpetuation of the disease by the seed tuber. The parent hill was partly diseased. This tuber unit appeared healthy on July 6, 1923, when other tuber units were obviously rugose mosaic (D), but the apparently healthy leaves developed spotting, streaking, and leaf-dropping, and the upper leaves showed typical rugose mosaic symptoms throughout by July 30 when this photograph was taken

D.—Green Mountain tuber unit completely rugose mosaic. This tuber unit was planted next to that of C in the same row, and was evidently diseased on July 6 when the other was still healthy. Note the greater dwarfing and the absence of leaf-dropping. Photographed on July 30, 1923, to the same scale as for D



(For explanatory legend see p. 518)

A.—On right, Green Mountain plant healthy except for current-season symptoms of rugose mosaic, indicated by the arrow. This is the twelfth hill from the east end of row 16 in section W–1 (fig. 1), as viewed from the south side. On left, healthy Green Mountain plant (thirteenth hill). Photographed on July 30, 1923. See Plate 8, C and D, for rugose mosaic from the seed tuber

B and C.—On right, Green Mountain plant with leaf-rolling mosaic perpetuated by the seed piece. On left, Green Mountain plant healthy except for current-season symptoms of leaf-rolling mosaic, indicated by arrow. Photographed on July 30, 1923. For a healthy control see Plate 9, A, on left

This is also indicated by the spread to the second row. There was no shoot contact early enough to permit the possibility of transmission by such means. That the disease spread in the plot in 1923 and was not perpetuated in the tubers from 1922 is indicated by the difference between the first and second rows on the north and by the absence of any rugose mosaic in the third row on the north and also nine rows (fig. 1, sec. W–6) planted elsewhere with tubers from the same healthy stock. Furthermore, other parts of the same healthy stock that were inoculated artificially as early in 1923 as diseased plants were available became diseased at about the same time and in the same way, except with a higher percentage of incidence.

Whether rugose mosaic was spread here by means of flea beetles (*Epitrix cucumeris* Harris), tarnished plant bugs (*Lygus pratensis* L.), or aphids, is open to question. *Aphis abbreviata* was observed here at the time of securing the inoculum for the artificial inoculations. However, roguing in another seed-plot, somewhat isolated, before any aphids were present and while flea beetles and tarnished plant bugs were present, did not result in the elimination of mosaic of the mild type. Transmission by flea beetles (*Psylloides affinis*), capsid bugs (*Calocoris bipunctatus*), and jassids (*Typhlocyba Ulmi*) has been demonstrated for leaf-roll by Murphy.[18]

In the same disease propagation plot there was also some spread of leaf-rolling mosaic, but only to the adjoining healthy row on the north, with the symptoms (pl. 9, B and C) appearing later than for rugose mosaic, in accordance with the results described previously, showing that rugose mosaic, leaf-rolling mosaic, and mild mosaic have progressively longer incubation periods. Contrary to what might be expected from these differences in the length of the incubation period, experience has shown that rugose mosaic, leaf-rolling mosaic, and mild mosaic are progressively more easily transmitted in the field from diseased to healthy plants (see Tables I and II).

These facts not only indicate that three types of mosaic may be spread in the field, but also confirm the theory previously advanced[19] that rugose mosaic may appear as partial infection of hills and tuber units as the result of uncontrolled field transmission occurring during the same season. Such current-season symptoms, as well as the incomplete or partial infection of the tuber, followed by a delayed manifestation of symptoms or by incomplete infection, often include spotting, streaking, and leaf-dropping (pl. 8, C and D).

TEST OF MOSAIC PERPETUATION THROUGH TRUE SEEDS

In 1921 the tubers and seed balls were saved together from each of a number of Green Mountain hills in a commercial field which contained about 5 per cent mild mosaic and which was infested with aphids. Some of the hills in question were mosaic and some were apparently healthy. The tubers were planted in the Orono, Me., greenhouse during the following winter and the seeds were planted in the same place in steam-sterilized soil. Seedlings were started in the winters of 1921–22 and 1922–23 and their first tubers were planted to produce a second generation. Of the original field-selected hills, hills 1 and 2 were mosaic in the field and produced, respectively, 2 and 3 mosaic tubers, and 7 healthy seedlings and no seedlings; hills 3 and 4 produced, respectively, 2 and 1 mosaic tubers, and 27 and 40 healthy seedlings; hill 5 produced 1 mosaic and 2 healthy tubers, and 6 healthy seedlings; hill 6 produced 2 healthy tubers and 32 healthy seedlings. Most of the seedlings in the second generation resembled Green Mountains closely, and were healthy in regard to the foliage. In brief, 1 mosaic plant (hill 1) produced 7 healthy seedling progeny while 3 apparently healthy plants (hills 3, 4, and 5) contracted mosaic which showed in their tuber-progeny but not in any of their 83 seedling-progeny.

EFFECT OF DIFFERENT CLIMATIC CONDITIONS IN 1922 ON MOSAIC LOTS FROM THE SAME TUBERS, AS SHOWN BY THE PROGENY GROWN IN NORTHEASTERN MAINE IN 1923

In order to ascertain the effect of climatic conditions on mosaic, seed pieces from the same tuber were planted in 1922 in three regions, namely, on Aroostook Farm in northeastern Maine, at Riverhead, Long Island, and at Nor-

[18] MURPHY, P. A. INVESTIGATIONS ON THE LEAF-ROLL AND MOSAIC DISEASES OF THE POTATO. Jour. Dept. Agr. and Tech. Instr. Ireland 23: 20–34, illus., 1923.
——— ON THE CAUSE OF ROLLING IN POTATO FOLIAGE; AND ON SOME FURTHER INSECT CARRIERS OF THE LEAF-ROLL DISEASE. Sci. Proc. Roy. Dublin Soc. (n. s.) 17: 163–184, illus., 1923.

[19] SCHULTZ, E. S., and FOLSOM, D. Op. cit. p. 97

folk, Va. Thirty mild mosaic Bliss Triumph tubers and a similar Green Mountain lot were represented in each place. As previously reported,[20] observations were made in 1922 by the same person, one of the writers, in the three localities. The results of these observations indicated that mottling was less distinct on the foliage at Norfolk than in the other localities. It was also noted that most of the vines in the three localities showed mild mosaic symptoms; a few plants plainly disclosed mosaic-dwarf or bad mosaic symptoms. However, mosaic-dwarf appeared on the plants from the same tuber in each of the three localities and no tuber produced plants having mild mosaic in one locality and a different stage of mosaic in another locality. The tubers from each hill were kept separate and in 1923 were planted on Aroostook Farm where the results presented in Table X were obtained. Tuber decay in the Green Mountain series made comparison possible only in the Bliss Triumphs.

The data in Table X show that rugose mosaic was contracted by 12 of the hills in Virginia, by 7 in Long Island, and by 3 in Maine. Leaf-roll was contracted by 8 hills in Virginia and by 2 in Long Island. Spindle tuber was contracted by 21 hills in Virginia and by 4 in Long Island and in Maine. Mild mosaic probably was present in more hills in the Virginia lot than is apparent from Table X, because it would be masked, if present, in the hills with rugose mosaic.

EFFECT OF FOUR REGIONS ON THE DISSEMINATION OF MOSAIC, LEAF-ROLL, AND SPINDLE TUBER IN FIVE VARIETIES

In a previous publication [21] evidence was recorded indicating that the dissemination of insect-borne diseases both differed between localities and varied in the same locality, and that such lack of uniformity in dissemination apparently was due to differences and variations in the number, kind, and seasonal

TABLE X.—*Effect of different climatic conditions in 1922 on mosaic lots from the same tubers, as shown by the percentage of disease in the progeny grown in northeastern Maine in 1923*

Series No.[a]	Mild mosaic			Rugose mosaic			Leaf-rolling mosaic			Leaf-roll			Spindle tuber		
	Va.	L. I.	Me.	Va.	L. I.	Me.	Va.	L. I.	Me.	Va.	L. I.	Me.	Va.	L. I.	Me.
1	------	100	100	50	12	------	------	------	------	50	75	------	------	------	------
2	------	------	------	------	------	------	------	------	------	------	------	------	100	100	100
3	33	100	100	55	------	------	------	------	------	------	------	------	100	50	------
4	75	75	100	25	25	------	------	------	------	------	------	------	75	------	------
5	------	------	100	------	60	------	------	50	------	100	------	------	100	------	------
6	40	------	------	------	------	------	------	------	------	------	------	------	80	------	------
7	100	100	100	35	------	------	------	------	------	------	------	------	100	------	------
8	42	------	100	------	------	------	------	------	------	------	------	------	100	------	------
9	------	100	100	------	------	------	------	------	------	------	------	------	100	------	22
10	------	100	100	100	------	------	------	------	------	------	------	------	88	------	------
11	------	------	------	75	50	------	------	------	------	100	------	------	(c)	------	------
12	------	------	------	100	50	100	------	------	------	------	------	------	------	------	------
13	------	100	100	------	------	------	------	------	------	------	------	------	100	------	------
14	------	------	100	100	------	------	------	------	------	100	------	------	------	100	------
15	------	------	------	------	------	------	------	------	------	------	------	------	100	75	------
16	------	100	100	------	------	------	------	------	------	44	------	------	100	------	------
17	------	100	100	24	------	------	------	------	------	------	------	------	------	------	66
18	100	100	100	------	11	------	------	------	------	------	------	------	90	------	------
19	------	100	100	------	------	------	------	------	------	------	------	------	100	------	------
20	------	100	100	36	------	------	------	------	------	100	------	------	100	------	------
21	------	100	100	100	------	------	------	------	------	------	------	------	100	------	------
22	------	------	------	------	------	------	------	100	100	18	------	------	------	------	------
23	100	------	100	------	------	------	------	------	------	------	------	------	------	------	------
24	18	100	100	------	------	------	------	------	------	------	------	------	81	------	------
25	100	100	100	------	------	------	------	------	------	32	------	------	------	------	81
26	------	100	------	------	------	------	45	------	------	------	------	------	50	------	------
27	100	100	100	------	------	------	------	------	------	------	------	------	100	------	------
28	------	100	100	------	16	------	100	------	------	------	------	------	66	------	------
29	------	100	100	11	------	9	33	------	------	------	------	------	------	------	------
30	100	------	------	------	------	50	------	------	------	------	27	------	------	------	------

[a] Each series consisted of seed-pieces grown from the same tuber in 1922.
[b] Labels in series 1, 11–17 for Virginia were defaced, but sacks were taken in order of packing.
[c] Partly diseased.

[20] SCHULTZ, E. S., and FOLSOM, D. Op. cit. p. 100.
[21] SCHULTZ, E. S., and FOLSOM, D. Op. cit. p. 102, 109.

duration of certain insects known to be capable of the transmission of these diseases. In order to secure additional evidence regarding the effect of different regions on the dissemination of mosaic, leaf-roll, and spindle tuber, the following test was carried on.

In 1922 comparatively healthy commercial stocks of five varieties, Green Mountain, Rural New Yorker, Rose Four, Irish Cobbler, and Bliss Triumph, were grown in four regions: On Aroostook Farm in northeastern Maine, on Highmoor Farm in southwestern Maine, at Riverhead, Long Island, and at Norfolk, Va. In each variety one original stock was selected and divided into ten 30-pound lots. Two such lots of each variety were planted in each locality, one lot between rows of Green Mountains with 100 per cent both mosaic and spindle tuber and the other lot between rows of Irish Cobblers with 100 per cent leaf-roll and the same per cent spindle tuber. In addition, at Riverhead and Norfolk a third lot of each variety was grown several rods from any 100 per cent diseased stock. The diseased Green Mountains also were originally from one lot which was divided for planting in the four regions; the same is true of the diseased Irish Cobblers. In 1922 representative samples of the 50 different healthy lots and of the diseased lots were kept, and in 1923 were grown on Aroostook Farm, where the observations recorded in Table XI were made.

The data in Table XI can be used for several kinds of comparisons. Mild mosaic was present in about 20 to 30 per cent of the hills in the Green Mountain lots from between leaf-roll Irish Cobbler rows in the four regions, and from the isolated lots not grown between diseased rows. Relative to the preceding, there was a somewhat higher percentage in the Green Mountain lots from between mosaic Green Mountain rows in northeastern Maine and southwestern Maine, and still more from the same in Virginia and Long Island. Mild mosaic was present in about 30 to 40 per cent of all the Bliss Triumph lots except a very small, unrepresentative one where 3 of the 5 plants were leaf-roll. Mosaic, that is not of the rugose type, probably was present in the lots of the other varieties originally, and was unusually conspicuous in 1923 when present. Except where masked by rugose mosaic, it was present in about 30 to 50 per cent of the hills in the Rural New Yorker lots, in about 10 to 20 per cent of the Irish Cobbler lots, and in about 30 to 60 per cent of the Rose Four lots. Therefore, with these types of mosaic there was no marked difference between different regions or between different plots in the same region.

Rugose mosaic, however, was much more abundant in the Virginia lots than in the corresponding ones from other regions, in each variety and in all three locations in the field relative to diseased rows. The only exception is in a small lot of three plants, which is negligible. Rugose mosaic was present, though in small percentages, in a majority of the lots from northeastern Maine and Long Island but was absent from most of the lots from southwestern Maine. It was present in all Irish Cobbler lots except one, though this variety showed the lowest percentages, and absent from all Rural New Yorker lots except those from Virginia. Therefore rugose mosaic was contracted only slightly in southwestern Maine but markedly in Virginia, and was contracted by different varieties in different amounts.

Leaf-roll was in 100 per cent of every Virginia lot from between leaf-roll Irish Cobbler rows (pl. 10, A) and for the lots from Long Island ranged from 51 to 93 per cent (pl. 10, B), from southwestern Maine from 0 to 60 per cent (pl. 10, C), and from northeastern Maine from 0 to 22 per cent (pl. 10, D), with averages respectively of 74, 26, and 6 per cent. It is clear that the spread of leaf-roll was greater as the region was farther south. Farther spread than from diseased rows to adjoining healthy rows occurred in Virginia and Long Island but not in either part of Maine. In Virginia the Green Mountains and Bliss Triumphs between the mosaic Green Mountain rows contracted over 90 per cent leaf-roll, and where grown a few rods from 100 per cent diseased rows contracted over 80 per cent in that region. Where there was a difference among the varieties Green Mountains and Bliss Triumphs generally were the most infected by leaf-roll.

Spindle tuber was originally in 100 per cent of the hills in both the diseased Green Mountain lots, the diseased Irish Cobbler lots, and the "healthy" Rose Four lots. The last will be disregarded in the comparisons to be made. From between the Green Mountain rows the Virginia lots ranged from 68 to 92 per cent spindle tuber, the northeastern Maine lots from 30 to 90 per cent, the Long Island lots from 4 to 78 per cent, and the southwestern Maine lots from 4 to 38 per cent, with respective averages of 85, 54, 39, and 16 per cent. From between the Irish Cobbler rows the

TABLE XI.—*Effect of different regions on dissemination of mosiac, leaf-roll, and spindle tuber in different varieties*

Variety and region	Location in field, 1922	Disease in 1923					
		Total number hills July 22, 1923	Mosaic not of rugose type [a]	Rugose mosaic	Leaf-roll	Spindle tuber	Total disease percentages
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Green Mountain:							
N. E. Maine	Between rows of Green Mountains with mosaic and spindle tuber.	213	31	1	1	45	78
S. W. Maine	do	149	32			7	39
Long Island	do	407	57	4	37	4	102
Virginia	do	[b] 377	41	22	99	92	254
N. E. Maine	Between rows of Irish Cobblers with leaf-roll and spindle tuber.	188	20	1	2	32	55
S. W. Maine	do	110	16		47	7	70
Long Island	do	471	31	2	70	58	161
Virginia	do	[c] 289	25	20	100	98	243
Long Island	Not between 100 per cent diseased rows.	593	20	6	53	40	119
Virginia	do	[c] 240	23	42	81	78	224
Rural New Yorker:							
N. E. Maine	Between rows of Green Mountains with mosaic and spindle tuber.	196	50			50	100
S. W. Maine	do	123	40			4	44
Long Island	do	341	46		11	50	107
Virginia	do	220	27	22	37	88	174
N. E. Maine	Between rows of Irish Cobblers with leaf-roll and spindle tuber.	143	29		2	34	65
S. W. Maine	do	103	33			18	51
Long Island	do	264	25		67	48	140
Virginia	do	[c] 28	4	73	100	85	262
Long Island	Not between 100 per cent diseased rows.	237	46		36	24	106
Virginia	do	68	19	13	20	36	88
Irish Cobbler:							
N. E. Maine	Between rows of Green Mountains with mosaic and spindle tuber.	218	15	2		90	107
S. W. Maine	do	77	14	4		38	56
Long Island	do	242	7	2	38	78	125
Virginia	do	631	12	12	16	92	132
N. E. Maine	Between rows of Irish Cobblers with leaf-roll and spindle tuber.	203	12	3	2	96	113
S. W. Maine	do	41	17	2	19	34	72
Long Island	do	214	17		89	76	182
Virginia	do	[c] 80	5	11	100	100	216
Long Island	Not between 100 per cent diseased rows.	192	20	1	17	74	112
Virginia	do	621	11	22	15	72	120
Bliss Triumph:							
N. E. Maine	Between rows of Green Mountains with mosaic and spindle tuber.	204	35			30	65
S. W. Maine	do	6	33			16	49
Long Island	do	263	43	4	74	22	143
Virginia	do	[c d] 212	24	45	93	68	230
N. E. Maine	Between rows of Irish Cobblers with leaf-roll and spindle tuber.	251	32			6	38
S. W. Maine	do	5			60		60
Long Island	do	255	29	4	93	8	134
Virginia	do	[c] 205	31	33	100	32	196
Long Island	Not between 100 per cent diseased rows.	233	40	2	38	6	86
Virginia	do	[c] 148	32	22	84	18	156
Rose Four:							
N. E. Maine	Between rows of Green Mountains with mosaic and spindle tuber.	158	22	5		(e)	27
S. W. Maine	do	94	48			(e)	48
Long Island	do	256	38	9	6	(e)	53
Virginia	do	[c] 124	19	25	30	(e)	74
N. E. Maine	Between rows of Irish Cobblers with leaf-roll and spindle tuber.	112	54	1	22	(e)	77
S. W. Maine	do	50	52		6	(e)	58
Long Island	do	262	38	10	51	(e)	99
Virginia	do	[d] 3	33		100	(e)	133
Long Island	Not between 100 per cent diseased rows.	283	58	1	7	100	166
Virginia	do	[c] 67	31	10	37	100	178

[a] Mild mosaic in the Green Mountains and probably in the Bliss Triumphs, and of undetermined type in the other varieties.

[b] All small except three healthy hills.

[c] All small.

[d] Many missing hills.

[e] Not examined because completely diseased in 1922.

Virginia lots ranged from 32 to 100 per cent, the Long Island lots from 8 to 76 per cent, the northeastern Maine lots from 6 to 96 per cent, and the southwestern Maine lots from 0 to 34 per cent, averaging, respectively, 79, 48, 42, and 15 per cent. Evidently spindle tuber increased more in Virginia than elsewhere, and less in southwestern Maine. A comparison of varieties as to susceptibility can not be made because the Green Mountains were the only one of the "healthy" stocks to be originally free from spindle tuber.

The total disease percentages are higher for Virginia in every variety whether from between diseased Green Mountain rows or from between diseased Irish Cobbler rows. For all varieties the total for Virginia is about double that of northeastern Maine, about triple that of southwestern Maine, and about half again greater than for Long Island. The total for Virginia probably should be larger because of mild mosaic being masked by rugose mosaic. It may be pointed out here that the test in 1922 in northeastern Maine was carried out in a field containing many plots with high percentages of disease of various types; in southwestern Maine in an isolated plot with the climatic conditions unusually unfavorable for growth; and on Long Island and in Virginia in plots surrounded by commercial varieties.

A few definite conclusions can be drawn from the preceding comparisons. Proximity to mild mosaic rows in 1922 helped to increase the amount of mild mosaic contracted by Green Mountains, more in Virginia and on Long Island than in Maine. Rugose mosaic was contracted markedly in Virginia but not elsewhere. While the "healthy" stocks, exclusive of the Green Mountains, were originally more or less infected with the milder types of mosaic and with spindle tuber, they were free from leaf-roll. The spread of this disease was greater farther south, and in Virginia and on Long Island was not restricted to spread from leaf-roll rows to adjoining rows. Spindle tuber increased more in Virginia than elsewhere. The total disease percentage increased most in Virginia and least in Maine.

One practical application of these conclusions is that the frequent statement to the effect that a hot climate causes rapid degeneration of potatoes, need not assume a direct effect, since an indirect effect by increasing the spread of degeneration diseases is now known to exist. It is also now more explicable why commercial stocks in northeastern Maine that are found to contain considerable leaf-roll usually can be traced back to a more southern source. It is plainly probable that the mosaic familiar to southern buyers of potato seed is not of the same type as that most often found in Maine. Emphasis is here given to the claim previously made [22] that the degeneration problem "because of its complexity may vary greatly from one locality to another" so that "control measures must be worked out for different sets of conditions, following research based initially on the general principles now fairly well understood."

A satisfactory explanation of these results would require more detailed study than has been made of each region relative to the presence of the various degeneration diseases in weeds and in neighboring commercial stocks, to the kinds, numbers, and development of infestations of transmitting insects, and to the effects of the diseases upon the development and maturing of the different varieties. Improvement should be made upon the methods used in making this test, especially in regard to original freedom from disease except for the one or two desired in any diseased stock. The requisite control measures to accomplish this are not yet fully understood.

SUMMARY

(1) Previously reported results with at least seven distinct degeneration diseases of potatoes suggest several new problems. A review of the symptoms includes several new ones, namely, spindling sprouts as an occasional symptom of leaf-roll even when unaccompanied by net necrosis, streaks and spots on corollas as symptoms of streak, and tuber cracking as a current-season symptom of unmottled curly dwarf inoculation. A fourth type of mosaic, "crinkle mosaic," is also distinguished tentatively; this is unrelated to "crinkle."

(2) Leaf-mutilation inoculations made in the field within the Green Mountain variety caused infection with mild mosaic, leaf-rolling mosaic, rugose mosaic, spindle tuber, unmottled curly dwarf, and streak, and also with various combinations of these diseases. This was followed by their natural spread from inoculated hills to adjacent hills, especially by mild mosaic.

[22] SCHULTZ, E. S., and FOLSOM, D. Op. cit., p. 112.

(3) Intervarietal leaf-mutilation inoculations of Green Mountains transmitted mild mosaic and crinkle mosaic from Bliss Triumphs; leaf-rolling mosaic, rugose mosaic, spindle tuber, and unmottled curly dwarf from Rurals; rugose mosaic from seedling lots; and leaf-rolling mosaic, rugose mosaic, and spindle tuber from Irish Cobblers. Varietal modification of symptoms was disclosed, mottling especially being suppressed by Rurals and Irish Cobblers.

(4) Intervarietal leaf-mutilation inoculations in insect cages showed that a repetition of the inoculation favors infection with mild mosaic and spindle tuber; that every one of several varieties was susceptible to spindle tuber; that Irish Cobblers either are symptomless carriers of mild mosaic or are resistant or immune to this disease; that "mosaic dwarf" sometimes is a combination of rugose mosaic, spindle tuber, and streak; that there is a varietal difference in susceptibility to streak; and that with conditions apparently similar not only will results of inoculations sometimes be negative where experience would lead one to expect infection, but there even may be a separation of viruses originally in combination in a given inoculum.

(5) Aphids sometimes do not transmit disease under conditions that apparently are the same as those giving positive results.

(6) With regard to current-season symptoms, a progressively smaller amount of infection was induced by leaf-mutilation inoculation with the leaflets, entire shoots, stems and petioles together, seed tubers, and roots, respectively, of rugose mosaic plants. Similar inoculation with juice from mild mosaic Green Mountain shoots and from certain mottled Irish Cobbler shoots, and the use of the latter in grafts, produced no apparent effects. Inoculation with the seed-cutting knife gave negative results with seven diseases and several combinations.

(7) Leaf mutilation inoculations in insect cages produced current-season symptoms in Green Mountains with progressively shorter incubation periods for mild mosaic, leaf-rolling mosaic, and rugose mosaic, but only rugose mosaic induced current-season symptoms in Rural New Yorkers and Irish Cobblers. Since these varieties are susceptible to leaf-rolling mosaic, this shows for this disease either a longer incubation period or a lower degree of varietal susceptibility correlated with varietal suppression of mottling.

(8) Aphid inoculations in insect cages produced current-season symptoms in Green Mountains with four types of mosaic—mild, leaf-rolling, rugose, and crinkle mosaic. Here the current-season symptoms of rugose mosaic following aphid inoculation were less marked than after parallel leaf-mutilation inoculations, which was also true in Rural New Yorkers and Irish Cobblers. Mild mosaic in at least the eighth consecutive generation of a Green Mountain strain was still mild and was distinct from rugose mosaic and the other types, contrary to prevailing theories of progressive increase in severity that apparently are based on general field observations made where the more severe types can increase. Facilitation of experimentation is obtained from the use of insect cages with openings in the top.

(9) Other aphid inoculations produced, in addition, current-season symptoms of three disease combinations—rugose mosaic and spindle tuber, crinkle mosaic and spindle tuber, and leaf-rolling mosaic and leaf-roll.

(10) Root and foliage contact with spindle tuber plants under insect-free greenhouse conditions resulted in no transmission. In another greenhouse, contact of roots and leaves, and of leaves in the absence of aphids and leaf mutilation but in the presence of other kinds of insects, was accompanied by the dissemination of mosaic but not of leaf-roll.

(11) A third species of aphids, the "buckthorn aphid" (*Aphis abbreviata* Patch), has been found to be common on potatoes and to be capable of transmitting mild mosaic, at least. Its early appearance has been accompanied by

EXPLANATORY LEGEND FOR PLATE 10

A.—The two middle rows are progeny of Green Mountains planted in Virginia between leaf-roll rows (left) and between mosaic rows (right). Photographed on August 18, 1923. For disease percentages see Table XI. For progeny of originally similar lots planted elsewhere see Plate 10, B, C, and D

B.—The two middle rows are progeny of Green Mountains planted on Long Island between leaf-roll rows (right) and somewhat isolated (left). Photographed on August 18, 1923. For disease percentages see Table XI. For progeny of originally similar lots planted elsewhere see Plate 10, A, C, and D

C.—The middle row is progeny of Green Mountains planted on Highmoor Farm in southwestern Maine between leaf-roll rows (in foreground) and between mosaic rows (beyond stake). Photographed on August 18, 1923. For disease percentages see Table XI. For progeny of originally similar lots planted elsewhere see Plate 10, A, B, and D

D.—The middle row beyond the gap in the foreground is progeny of Green Mountains planted on Aroostook Farm between leaf-roll rows. Photographed on August 18, 1923. For disease percentages see Table XI. For progeny of originally similar lots planted elsewhere see Plate 10, A and C

(For explanatory legend see p. 526)

the natural spread of rugose mosaic and leaf-rolling mosaic with the appearance of current-season symptoms. These include spotting, streaking, and leaf dropping, which also may result from delayed symptoms or incomplete infection which sometimes follow tuber perpetuation.

(12) Ninety healthy seedlings were grown from seeds produced by several Green Mountain plants, although the parent plants either were affected with mosaic or had mosaic tuber progeny.

(13) Mild mosaic Bliss Triumph sister hills (from the same seed tubers) planted in three regions showed less distinct mottling in Virginia but contracted more rugose mosaic, leaf-roll, and spindle tuber there than on Long Island and contracted least of all in northeastern Maine, as shown by the progeny all grown in northeastern Maine.

(14) Comparatively healthy commercial stocks of five varieties divided and grown in four regions mostly next to diseased rows, with the progeny all grown in one place, showed that mild mosaic spread more in Virginia and on Long Island than in southwestern and northeastern Maine; that rugose mosaic was contracted markedly in Virginia but not elsewhere; that leaf-roll spread more farther south; that spindle tuber increased more in Virginia than elsewhere; and that the total disease percentage increased most in Virginia and least in Maine. It is therefore concluded that a hot climate may cause degeneration of potatoes indirectly through favoring the spread of diseases. It also suggests why northern-grown seed is preferred; how buyers and sellers of seed if from different regions may misunderstand each other in discussing "mosaic," because of familiarity with different types; and why the potato degeneration problem requires local or regional study.

STUDIES OF SPORE DISSEMINATION OF VENTURIA INAEQUALIS (CKE.) WINT. IN RELATION TO SEASONAL DEVELOPMENT OF APPLE SCAB [1]

By C. N. FREY,[2] *formerly Assistant in Plant Pathology, University of Wisconsin*, and G. W. KEITT, *Professor of Plant Pathology, University of Wisconsin*

During the season of 1916 it became apparent to the junior author, who was then beginning work on the control of apple scab,[3] that further studies of certain details of the life history of the causal fungus in relation to the seasonal development of the disease under local conditions were fundamental to the satisfactory application of control measures. A clearer understanding of certain aspects of spore dissemination and infection appeared to be of primary importance. Accordingly, in the spring and summer of 1917, some studies of these questions were undertaken as a minor problem in such time as the writers could devote to it. The advent of the war prevented continuation of this work, and subsequently pressure of duties in another field upon the senior author has prevented earlier publication of the results. However, the junior author and his coworkers have extended these investigations and plan to supplement the following account in a later paper.

A STUDY OF THE SPORE CONTENT OF ORCHARD AIR

METHODS

Of the various methods for the study of spore dissemination described in the literature with which the writers are conversant, none appeared exactly to meet the requirements of their problem. Consequently an attempt was made to devise one.

ELECTRICAL PRECIPITATION

At the suggestion of L. F. Hawley of the Forest Products Laboratory of the United States Department of Agriculture, an attempt was made to adapt to the purposes of the present study the electrical method described by Cottrell [4] for removing suspended particles from gases. Preliminary experiments showed that when spores of *Venturia inaequalis*, *Ustilago zeae*, or certain other organisms were brought within the field of a static machine most of them became negatively charged and passed to the positive pole. A few of the spores tested became positively charged, a small number of each kind always passing to the negative pole.

These observations encouraged the writers to pursue their experiments somewhat further. A glass tube 12 inches long and 1.5 inches in diameter was set up between the poles of the static machine. In each end of this tube was placed a rubber stopper through which ran two glass tubes, each 2 inches long and one-fourth inch in diameter. One of these tubes was attached to a vacuum pump, while the opposite one was left open to permit the access of air. A copper wire was passed through the other two tubes in such manner as to run through the middle of the larger tube and to attach at one end to the negative pole of the static machine. All openings into the larger tube were sealed with wax, with the exception of the two small tubes to permit the ingress and egress of air. Tin foil was lightly wrapped around the outside of the large tube and connected with its inner wall. A copper wire attached to the foil was connected with the positive pole of the static machine. A thin coat of vaseline had previously been rubbed on the inside of the tube to aid in holding the spores as they were precipitated against it. Air currents bearing abundant fungus spores were then passed through the tube while the static machine was in operation. When the current of air was suitably regulated and the static machine delivered a high potential, about 30,000 to 50,000 volts, most of the spores were caught on the first 3 or 4

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[3] A comprehensive account of this disease and its causal fungus, *Venturia inaequalis* (Cke.) Wint., with an extensive bibliography, is given in the following citation: WALLACE, E. SCAB DISEASE OF APPLES. N. Y. Cornell Agr. Exp. Sta. Bul. 335, p. 545–624, illus. 1913.

[4] COTTRELL, F. G. THE ELECTRICAL PRECIPITATION OF SUSPENDED PARTICLES. Jour. Indus. and Engin. Chem. 3 : 542–550. illus. 1911.

inches of the tube, being deposited in a thin film on its inner wall. When smut spores were used the tube was quickly coated with a black layer, the darkest zone being situated near the inlet, showing that they were precipitated very promptly upon entering the charged field. When the apparatus was suitably adjusted very few spores passed through the tube. This was determined by passing the outgoing air through a wash bottle containing oil and water and examining the fluid microscopically. Only occasional spores were found.

Preliminary steps were then taken toward developing an electrical precipitation apparatus which might be operated satisfactorily under orchard conditions. However, pressure of work, expense of the electrical equipment necessary, and the fact that very satisfactory results were being obtained by the method described below led to the discontinuance of experiments with the electrical method. The writers believe, however, that this method has possibilities of adaptation to problems involving a study of the spore-content of air. The results obtained appear to be in general agreement with those of Buller (p. 192–195) [5], who has studied the electrical charges on spores from a different point of view.

FILTRATION ON MEMBRANES [6]

Early in the consideration of the problem it occurred to the writers that it might be possible to devise an apparatus which might be run continuously and so constructed that spores could be filtered from the air upon transparent or translucent membranes on which they might be counted under the microscope. Preliminary tests with membranes prepared from cloth, fiber, gelatin, nitrated cellulose, cellulose acetate, and viscose were unsuccessful. The three latter-named materials were not sufficiently porous to allow adequate passage of air. Pure filter paper was tried, and it was found that, by specially treating [7] soft Swedish filter paper, nearly pure cellulose, there was produced a tough, waterproof membrane which proved to be satisfactory. Some special Swedish filter papers and Whatman's hardened filter paper were then investigated and found to be sufficiently waterproof to resist disintegration when wet by rain. The paper, however, should not be so altered by treatment with acid that it loses its porosity and precludes adequate passage of air.

A motor-driven vacuum pump was then set up and a heavy-walled rubber tube was used to connect its intake with a special apparatus for holding the filter paper (fig. 1). It consisted of a thick-walled brass tube (A) on the machined end (B) of which was screwed a very closely fitting brass cap (C) in the middle of which was an aperture 1 inch in diameter. A disc (D) of filter paper of suitable diameter, underlain by a supporting disk of fine silk fabric of similar size, was placed upon the machined end of the metal tube, and the cap screwed tightly over it. In this way it was held firmly in position, the cap making a joint that was air-tight, while a circular area 1 inch in diameter was exposed for purposes of filtration. A perforated rubber stopper (E) connected the device with the suction tube by means of a glass tube. Numerous tests of the capacity of the machine with different filters of the type used throughout the experiments showed that approximately 1,200 liters of air passed through the apparatus in an hour.

The membranes were removed at intervals as circumstances warranted. As soon as a membrane was removed it was placed in a small Esmarck dish and treated with a small amount of glycerine to make sure that the spores remained in place. In each test a membrane similar to the one used for filtration was placed beside the latter, collected with it, and studied microscopically, as a control against chance accumulation of air-borne ascospores. Studies of these, however, showed so few spores that it seemed unnecessary to make any correction in the results for them. The number of spores caught on the membrane was determined by direct counts under the microscope. The olivaceous color and characteristic form and size of the spores made them easily observed without staining. If the number was

[5] BULLER, A. H. R. RESEARCHES ON FUNGI. v. 1, illus. London, New York, etc. 1909.

[6] In later studies the junior author and his coworkers have developed another filtration technique which is being used in their further work. Orchard air is drawn by means of a motor-driven suction apparatus through a suitably arranged filter of nitrocellulose. Thence it passes through a gas meter which records the volume. At the end of the run, the nitrocullulose filter, bearing the spores caught, is dissolved in a suitable glass container in a mixture of alcohol and ether and allowed to evaporate to a gel. The ascospores settle to the bottom and their number is computed on the basis of microscopic counts. This is an adaptation of a technique developed by Pasteur. It will be described in more detail in a later paper.

[7] The filter paper was dipped for about one-half minute in nitric acid, sp. gr. 1.42, transferred for a brief period to sulphuric acid (equal volumes of 1.84 sp. gr. sulphuric acid and water), and washed in water and then in a weak solution of ammonia.

small or nil the entire surface of the exposed area of the membrane was observed. This was accomplished and duplication of observation avoided by the use of a mechanical stage and a micrometer. If spores were numerous, counts were made of at least 30 fields, chosen at random but without duplication from all parts of the filter, and the total number was computed on the basis of this average. The results thus obtained agreed closely with those from counts over the entire exposed area. A Leitz microscope was used, with a No. 4 eyepiece, a No. 6 objective, and a tube length of 170 mm.

FIELD EXPERIMENTS

During late April and early May preliminary observations were made to determine the time at which ascospores would be sufficiently mature for natural discharge. Over-wintered apple leaves bearing numerous perithecia of *Venturia inaequalis* were brought into the laboratory daily, thoroughly moistened, and placed in moist chambers in such position that one could ascertain by miscroscopic examination of the glass surface below them whether or not ascospores had been discharged. No discharge from such material was observed until May 7.

On May 6, just as the cluster buds of the apple were beginning to open, the apparatus was installed in the Turville[8] orchard, about 1½ miles south of Madison. This orchard, consisting of about 50 large trees planted in four rows running east and west, was in sod, and the ground under and about the trees was abundantly littered with over-wintered leaves which bore perithecia of *Venturia inaequalis* in great profusion. The apparatus was placed under the outer branches of a Fameuse tree which stood in the southernmost row, second from the eastern end. The membrane was placed at elevations varying from 1½ to 3 feet above the ground, and was protected from rain by a small roof-like shelter which was raised sufficiently to permit free passage of air over the filter. The machine was run continuously, except for brief stops for changing membranes and caring for the motor, until June 19, and subsequently at intervals. The results are summarized in Table I in correlation with hourly records of rainfall. Hourly records of temperature and wind velocity show valuable correlations but are not included in the table because of limitations of space.

The field and laboratory observations indicate that on May 6, when the field filtration tests were started, the ascospores were just approaching maturity. Apparently no discharge had occurred prior to this time. During the dry period from May 6 to 18 no Venturia spores were caught. On May 19, rain fell from about 2 to 5

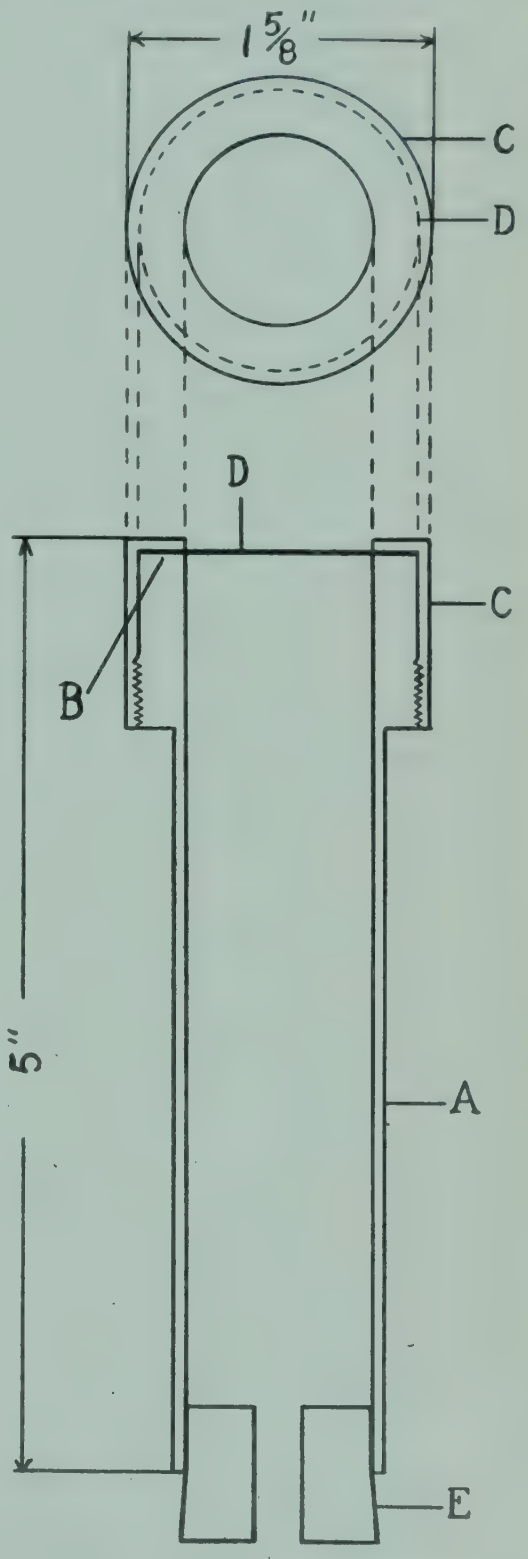
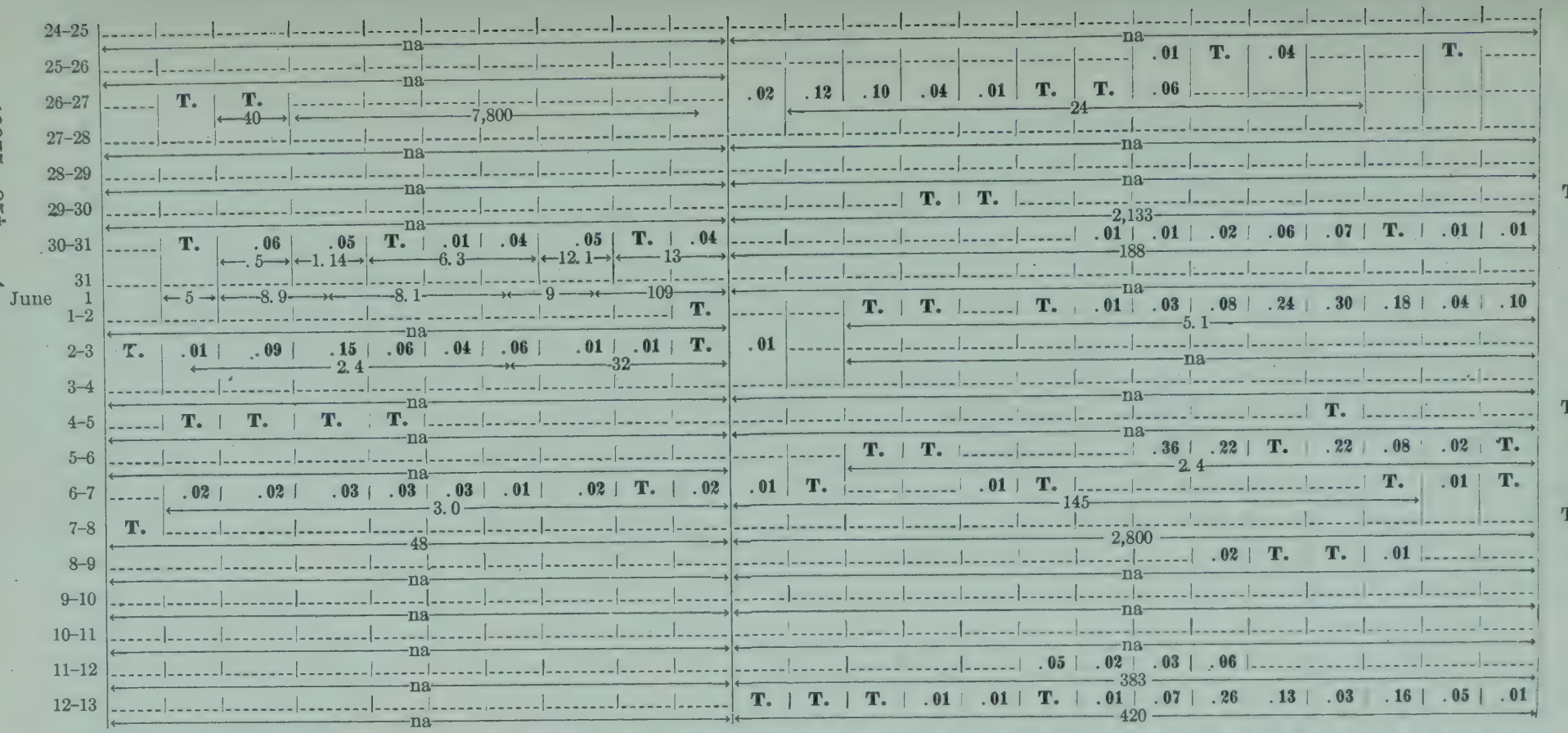


FIG. 1.—Device used for filtering spores from the air

[8] Grateful acknowledgments are made to Thomas and Wm. D. Turville for their kindness in making their orchard available for this work.

TABLE I.—*Results of filtration experiments, showing orchard air content of ascospores of V. inaequalis in relation to hourly records of rainfall, Madison, Wis., 1917*

Dates	Records by hours[a]																						Total rainfall (inches)
	A. M.				P. M.												A. M.							
	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	
May 5–6	na										na														0
6–7	na										na														0
7–8	na										na						T.					T.			T.
8–9	na										na														0
9–10	na										na														0
10–11	na										na														0
11–12	na										na														0
12–13	na										na														0
13–14	na										na														0
14–15	na										na														0
15–16	na										na														0
16–17	na										na														0
17–18	na										na														7
18–19	na										na														0
19–20	na																T.	.54		.23					.77
20–21	89			204							840							30							
21–22	na																					T.			T.
22–23	.01	.09	.23	.17	.16	.03	T.	T.			(?)[b]			T.	.11	T.	T.	T.	.08	.02	.01	.03	.05	.03	.33
23–24	.07	.04	.05	.03	.01								.01	.05	T.	.03	.05	.05	.07	.07	.04				1.06
	4										23														
	na										109														.20
	na										na														0



[a] In the space representing each hour, the upper line (the line of the date) is devoted to the rainfall record, expressed in inches. In order to make the rain periods stand out more sharply, zeros are omitted and bold-faced type is used. "T" indicates that only traces of rain fell.

The lower line gives the ascospore discharge record. The arrows indicate the periods during which the filter was operated. The numbers between the arrows represent the average number of liters of air filtered for each ascospore caught. *"na" indicates that no ascospores were caught during the period shown*, though air was filtered at the standard rate of approximately 1,200 liters per hour.

The data on rainfall are taken from the records of the Madison station of the U. S. Weather Bureau, which is situated on the top of a four-story building about 1½ miles from the Turville orchard. These data are therefore not to be interpreted as representing more than a close approximation of the conditions which obtained in the orchard. Inches of rainfall on the dates omitted from the table follow: June 20–21, 0.03; 22–23, 1.24; 23–24, 0.01; 24–25, 0.01; 25–26, 0.53; 27–28, 0.45; 28–29, 0.60; 29–30, 0.10; July 5–6, 0.03; 6–7, 0.07; 10–11, 0.03; 13–14, 0.15.

[b] The filter was so covered by sleet that no reliable record could be obtained.

TABLE I.—*Results of filtration experiments, showing orchard air content of ascospores of V. inaequalis in relation to hourly records of rainfall, Madison, Wis., 1917*—Continued

Dates	Records by hours																						Total rainfall (inches)
	A. M.				P. M.												A. M.							
	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	
June 13–14	T.	T.	.02								T.	.01													
14–15			T.	T.	T.			T.	T.	T.	T.	T.				.01									.03
15–16	na										137														.01
16–17	na										1,000														0
17–18	na										na														T.
18–19	na										na				T.	T.									0
19–20	na										na														0
30	na										na														0
July 1	na										na														0
1–2	na										na						.01	.08		.51	.02				62
2–3	na										30														0
3–4	na										na														0
11–12	na										na														0
12–13		.14			T.		T.			T.	.01	T.													.15
17–18	na										33														0
18–19								.07	.01			.02	T.												.10
19–20											.07	T.													.07
21–22	na										56														0
											na														.08
											na														1.28
																									1.36

a. m., and the first discharge of the season was recorded by the apparatus. Ascospores were caught at the average rate of 1 ascospore to 30 liters for the 3-hour period covered. From 8.30 to 10.30 a. m., while the leaves were still wet, the average rate of catch was 1 ascospore to 89 liters. From 10.30 a. m. to 6 p. m. it was 1 to 204 liters; and from 6 p. m., May 19, to 8 a. m., May 20, 1 to 840 liters. No spores were caught from 8 a. m. to 6 p. m., May 20, by which time the leaves had thoroughly dried.

During the night of May 20–21 rain or sleet fell almost continuously and rather high winds prevailed. Unfortunately, in spite of the shelter which had been erected to protect the membrane from weather, it was so washed by rain and sleet that no trustworthy record could be taken. In the morning, however, the winds became less violent. The rain continued from 9 a. m., when a new membrane was put on, until about 4 p. m. From 9 a. m. to 5.30 p. m. ascospores were caught at the average rate of 1 ascospore to 0.4 liters. This proved to be the heaviest discharge recorded during the experiments. The wind velocity for the period of this run varied from 14 to 23 miles an hour.

Rain began to fall about 11 p. m. May 21 and continued until about 1 p. m. May 22. From 7 p. m., May 21, to 7 a. m., May 22, ascospores were caught at the average rate of 1 ascospore to 23 liters. The wind velocity for the period during which this rain fell varied from 21 to 34 miles an hour (NE. to NW.). No record is available for the daylight hours of May 22. The machine ran from 6 p. m., May 22, to 8 a. m., May 23, and recorded an average catch of 1 ascospore to 109 liters. Apparently the leaves were sufficiently moist from the morning's rain to continue this light discharge. Following thorough drying the next morning, discharge ceased and no spores were caught until the next rain.

Light showers fell between 1 and 7 a. m., May 26, but no record for this period is available. From 10 to 11 a. m., however, during traces of rainfall, the machine was run, and ascospores were caught at the rate of 1 ascospore to 40 liters. From 11 a. m. to 5.30 p. m., with no additional rainfall, the catch was only 1 to 7,800 liters.

Showers fell during the night of May 26–27, and spores were caught at the rate of 1 ascospore to 24 liters. The wind velocity during this rain period varied from 10 to 22 miles.

No more ascospores were registered by the machine until the night of May 29–30, when, following traces of rain, they were caught at the rate of 1 ascospore to 2,133 liters. On May 30, however, an excellent opportunity for a fractional study of a discharge period presented itself. From 9 to 10 a. m. a trace of rain fell, followed by a fairly heavy rain from 10 to 12, a trace from 12 to 1, and almost continuous rain throughout the remainder of the afternoon. The wind velocity from 10 a. m. to 6 p. m. varied from 13 to 23 miles an hour. From 10 to 11 a. m. ascopores were caught at the average rate of 1 ascospore to 0.5 liters; from 11 to 12, 1 to 1.14; from 12 to 3, 1 to 6.3; from 3 to 4, 1 to 12.1; from 4 to 6, 1 to 13. From midnight until 8 a. m., May 31, it rained almost continuously. However, from 6 p. m., May 30, to 8 a. m., May 31, the average rate of catch was only 1 ascospore to 188 liters. The old leaves on the ground remained wet in the tall grass of the orchard until about 3:30 p. m., May 31. By this time the wind, which had freshened about 3 p. m., had begun to dry them perceptibly. This is very well reflected in the ascospore content of the air. From 9 to 10 a. m., May 31, spores were caught at the rate of 1 ascospore to 5 liters of air; from 10 to 11.30, 1 to 8.9; from 11.30 to 2.30, 1 to 8.1; from 2.30 to 3.40, 1 to 9; from 3.40 to 6, 1 to 109. It appears that, at the beginning of this rain period, there was an abundance of mature ascospores ready for discharge. During the first 2 hours (10 to 12 a. m., May 30) the rate of discharge was very high. Subsequently, however, it dwindled rapidly, and relatively few mature spores appear to have been available for discharge during the rain of the following night. The rate of discharge increased very markedly on the morning of May 31, probably due to the maturing of asci during the current moist period.

No further discharge was recorded until the rain of the night of June 1–2, when ascospores were caught at the rate of 1 ascospore to 5.1 liters (8 p. m. to 8 a. m.). From 9.30 a. m. to 2.30 p. m., June 2, it rained continuously, and ascospores were caught at the rate of 1 ascospore to 2.4 liters. From 2.30 to 6 p. m., although showers continued, the rate of catch fell to 1 to 32 liters. During the following night, no spores were caught. In this instance, the period of rapid discharge was considerably more prolonged than was the case in the preceding rain.

No further discharge was recorded until the rain of the night, June 5–6, when the average rate of catch from 8 p. m. to 8 a. m. was 1 ascospore to 2.4 liters. The rain continued through-

out the day of June 6, the catch from 9 a. m. to 6 p. m. being at the rate of 1 to 3 liters. During the following night, although there were slight showers, the rate fell to 1 ascospore to 145 liters. In this, as in the preceding instance, the period of rapid discharge was fairly prolonged, probably extending over about 15 hours. It appears likely that the more extended periods of these later heavy discharges, as compared with that of May 31, are to be attributed to a more generally advanced stage of maturity of asci and possibly to more rapid ripening.

Minor discharges occurred during the showers of June 7, 12, 13, 14, and 15. Although fairly heavy rains fell on June 12 and 13, the discharges were very light. It appears that the heavy discharges on and prior to June 7 went far toward exhausting the supply of ascospores. The failure to catch any spores whatever during the rainfall of 0.03 inch of June 9 is not understood. It is possible that there was less precipitation in the orchard than at the weather station. It is conceivable, on the other hand, that the supply of ripening asci was so nearly exhausted that no significant numbers were in condition to discharge at this time.

No spores were caught during the dry period from June 16 to 20.

Due to the absence of the senior author, no record was taken for the period of June 21 to 29.

Rain fell in the latter part of the night of June 30–July 1. From 6 p. m. to 8 a. m., the average rate of catch was 1 ascospore to 30 liters. When it is considered that most of the discharge of this period evidently occurred from about midnight to 5 or 6 a. m., it is apparent that this represents a fairly heavy discharge for so late a date.

During the dry period, July 2 to 4, no ascospores were caught.

The next run was made on the night July 11–12, when light showers fell from about 6 to 8 p. m. The catch was at the rate of 1 ascospore to 33 liters. From 8.30 a. m. to 6 p. m. July 12, there was no rain, and no spores were caught.

The next run was made on the night of July 17–18. Light showers fell between 7 and 9 p. m. From 6 p. m. to 8 a. m. the rate of catch was 1 ascospore to 56 liters.

Showers fell from 6 to 8 p. m., July 18. No ascospores were caught in a run from 6 p. m., July 18, to 8 a. m., July 19. During the following 24 hours, no rain fell and no spores were caught.

From 2 to 4 a. m., July 22, 1.36 inches of rain fell. From 6 p. m., July 21, to 8 a. m., July 22, no ascospores were caught. It appears that the season's supply was exhausted at this time.

From the results of these experiments it is readily apparent that the presence of an adequate amount of water is a primary requisite for the ejection of ascospores of *Venturia inaequalis*. Under the conditions of the tests, dew alone was not sufficient to occasion discharges of any considerable consequence. It is apparent, further, that rain water is very important, not only in relation to the discharge of ascospores, but for their maturation as well. This latter point has been more clearly established in later studies by the junior author and his coworkers and will be discussed in a later paper.

The records of May 31, June 3, and June 6–7 show that when ascospores in abundance are in condition to be ejected, heavy discharges occur at the beginning of a rain period and continue during continuous rain as long as the supply of ripe asci lasts. The periods of heavy discharge varied from several hours on May 31 to about 15 hours on June 3 and June 6–7.

The maximal average concentration of spores for a single run of the machine, 1 ascospore to 0.4 liters, was obtained between 9 a. m. and 5.30 p. m., May 21. When one considers that only traces of rain fell after 2 p. m., it appears likely that, at the period of maximal discharge, the concentration of spores may have been considerably greater than the average for the entire run. In order to gain a still more concrete conception of such concentrations of spores it seems to be of value to compute on the basis of the records just mentioned the number of ascospores which would have passed in the 8½-hour period studied through an imaginary orifice 10 cm. square interposed 3 feet above ground perpendicular to the wind direction. With the average wind velocity for this period of 18.5 miles an hour and the average ascospore content of the air 3 feet above ground of 1 ascospore to 0.4 liters, as shown by the records, it appears that over 6,300,000 ascospores would have passed through the orifice postulated.

In considering these records of ascospore discharge in relation to the seasonal development of the host plant and to control measures it would appear that they are in accord with the widely prevalent idea that the first ascospore discharges of *Venturia*

inaequalis are to be expected at about the time the fruit buds separate in the clusters just prior to the blooming period. Subsequent studies, however, have shown that, in the vicinities of Madison and Sturgeon Bay, Wis., ascospore discharge ordinarily begins at a much earlier stage of bud development and that the lateness of the initial discharge of 1917 may be attributed largely to the paucity of rainfall in early April and the first 18 days of May. It should be noted that even in 1917 it was shown by wetting leaves freshly collected from the orchard that asci were capable of discharging spores as early as May 7.

Although the air filtration experiments were planned primarily for the study of ascospore dissemination a careful watch was kept for conidia. After June 2, when the first scab lesions were observed, conidia began to appear in small numbers on the membrane in rainy periods, particularly when the rain was accompanied by high winds. They were never caught, however, except under these conditions. These facts led the writers to make certain observations upon the conditions which favor dissemination of conidia.

Apples leaves bearing abundantly sporulating scab lesions were placed in a glass tube through which a strong current of air was driven. A glass slide smeared with glycerine was held opposite the outlet of the tube in position to catch air-borne spores. Microscopic examinations following numerous repetitions of this test revealed only very small numbers of spores on the slides. Similar experiments with lesions on apple fruit gave like results. However, when these same lesions were treated with a fine mist of water applied by means of an atomizer, the droplets which accumulated contained conidia in great numbers. Similarly, when sections were cut from lesions with a dry razor and observed under the microscope, the addition of a droplet of water led to swelling of the conidiophores and immediate detachment of conidia. Furthermore, drippings collected during rains from scabby apple trees have shown an abundant content of conidia of *Venturia inaequalis*. These results, in conjunction with those from the air filtration experiments, indicate that no important dissemination of conidia is to be expected in the absence of water, though undoubtedly some spores are dislodged by wind-whipping of leaves, fruit, or branches, by contact with wind-blown particles, and in other minor ways. It appears, therefore, that the important agency for dissemination of these conidia is meteoric water moving under the influence of wind and gravitation.

GERMINATION TESTS

ASCOSPORES.—Germination tests with ascospores were made at frequent intervals throughout the period in which their natural discharge was observed in the field (May 18 to July 18). Fragments of leaves bearing perithecia were moistened and placed in such position that the spores would be discharged upon droplets of sterile distilled water on clean sterile glass slides in moist chambers or on plates of 2 per cent agar in water. The drops or plates were then placed in incubators at 18° to 25°C. In all cases naturally discharged ascospores showed a high percentage of vigorous germination, usually approximating 100 per cent. Germination tests of ascospores caught on the membranes gave like results. Some experiments conducted at constant temperatures ranging from 2° to 26° C. showed germination at all the temperatures tried, the optimum for germination and growth under these conditions being between 14° and 20° C.

CONIDIA.—Germination tests of conidia from fruit and leaf lesions were made at frequent intervals from their first appearance until late fall. The same media and incubators used for ascospore germination were employed. Abundant viable conidia could be found at any time during these experiments, but the percentage and vigor of germination varied greatly in individual tests. This is not surprising in view of the fact that mature conidia are much less protected than are ascospores and not so uniformly removed from the parent fungus when rain follows their maturity. Consequently, a sample of conidia, even when secured by touching a droplet of water borne in a wire loop to the sporulating surface of a scab lesion, is likely to be less uniform than a sample of naturally discharged ascospores.

SEASONAL DEVELOPMENT OF THE HOST PLANT

In relation to production and dissemination of spores and the seasonal development of the disease, the following brief notes on the seasonal development of the host plant are pertinent:

The unfolding of both vegetative and fruit buds was unusually late and slow in 1917. On May 7 the pink of the

petals of many fruit buds was evident, and by May 18 nearly all of the blossoms were open. On May 22 the largest leaves were about 2½ inches long, and petals were falling. The last leaves to develop appeared from about May 19 to 24, some variation occurring with varieties and individual trees. Most of the petals were off by May 25, and little leaf expansion occurred after June 2, when the first conidia of the season were observed. There was practically no leaf expansion after June 8 (except on "water sprouts").

SEASONAL DEVELOPMENT OF THE DISEASE

The seasonal development of the disease was studied by means of a series of bagging experiments, supplemented by daily observations in the orchard. No spray was used in this orchard. Each day from May 7 to June 19, six branches on at least two varieties were inclosed in large manila paper bags which were securely fastened in place with strong twine. In this way comparative records were kept of the development of the disease on branches which had been exposed to different infection periods. Many of the bags were broken [9] during storms, necessitating discarding a considerable number of branches from the experiment. Enough were left, however, to give some valuable data, a brief account of which follows.

None of the branches which were bagged before May 19 and on which the bags remained unbroken during the storms of May 30 and June 2 showed any evidence of infection throughout the period of observation, which ended on August 24. All branches bagged after May 22 developed infection, and with the exception of the last two leaves formed, which in some cases were put out after May 22, developed about as many leaf lesions as those which were continuously exposed to infection. Leaves of branches bagged after the rain on June 2 were infected in the same manner and to approximately the same extent as those of similar unbagged branches. This is of especial interest in view of the heavy ascospore discharge and favorable infection period of June 6–7.

The first leaf infection of the season was noted on June 2. This would allow a 14-day incubation period after the rain of May 19. This agrees with the results of field inoculation studies made in early spring in later years, in which the periods of incubation for leaf infection have commonly varied from 13 to 17 days. The records on ascospore dissemination and seasonal development of the disease are, therefore, in accord in placing the first ascospore discharge and infection of the season in the rainy period of May 19 to 22. The fact that no infection of significance occurred on leaves of branches which were bagged during the period May 19 to June 2 and subsequently exposed indicates that the leaves had become highly resistant to infection by the scab fungus by the latter date.

A striking suggestion of the variation in the susceptibility of leaves in relation to their stage of development was found in the number, distribution, and incubation periods of leaf lesions induced by natural infection. On June 2, when the first scab lesions of the season were observed, leaf expansion was almost finished. Lesions were clearly visible, in most cases, only on the upper surfaces of the apical leaves (for convenience called leaves No. 1) of shoots. Occasionally, early evidences of infection were apparent on the next leaf back, and within a day or two leaves No. 2 showed infection in about the same degree as No. 1. The development on leaves No. 3 and No. 4 was visible by June 8, while by June 12 to 15 small lesions were evident on many leaves No. 5 and No. 6 and rarely on No. 7. In late July and August the older leaves frequently showed traces of fungous growth on their lower surfaces but no definite lesions. The earlier infection thus appeared on the younger leaves and usually on the upper surfaces after relatively short incubation periods, while the latest infections to become evident appeared after prolonged incubation on the lower surfaces of the older leaves. Later field observations and field and greenhouse inoculation studies have confirmed the general outlines of these observations. They have shown, however, that there is a considerable range in the degree of these variations under different conditions. Under some conditions, for instance, only one or two leaves of a shoot will become infected from a given inoculation, while under others infection may develop on a half dozen or more.

The seasonal development of the disease on the fruit was much more difficult to follow in detail than on the leaves because of shedding induced by insect injury and other causes. The

[9] In later seasons the substitution of bags made of parchment paper obviated this difficulty.

first observation of fruit infection was made on June 8, when lesions were visible on the young fruits, calyx lobes, and pedicels. Daily field records showed that lesions continued to appear on the fruit throughout the period of observation, which ended on August 24. The results from the bagging work showed that the first fruit infection occurred during the rainy period of May 19 to 23. The most abundant infection and the largest lesions developed in the early part of the season. The fact that the later lesions are smaller suggests that the fruit, like the leaf, may develop resistance with age. Such resistance, however, appears to be developed in a much smaller degree, since fruit infection has been shown to appear late in the season or even in storage.

INOCULATION EXPERIMENTS

The life history studies were supplemented by some inoculation work in the orchard. Metal boxes, 28 by 12 by 10 inches, with tightly fitting, felt-lined covers, were constructed as infection chambers. A slit at one end of each box permitted a branch to enter and a tripod served as an adjustable support. The box was placed in position about the inoculated or control branch, the cover put in place, and the orifice through which the branch entered closed with plastic clay. A metal shelter about 4 inches above the box shaded it from the sun. When necessary the box was cooled by water.

Three types of shoots were selected for inoculation: (1) Those which had been bagged continuously since May 18 and were therefore free from infection, (2) those which had been exposed to infection throughout their development, and (3) those which developed during July ("water sprouts") and consequently bore young leaves. The inocula consisted of sterile-water suspensions of conidia from naturally infected leaves and from oatmeal-agar cultures. The experiments were performed in July and early August.

The chief results of these experiments may be summarized briefly. Thirty-six trials on disease-free shoots which had been bagged prior to May 18, and which bore no young leaves, gave no infection. In similar trials on shoots which had been exposed continuously to infection, there was no evidence that additional infection was induced. However, on branches which bore young leaves, abundant infection was induced on the young leaves provided the temperature was kept from running too high. While the exact maximum temperature for infection was not determined it appeared to be not much higher than 25° C. The maximum age of susceptible leaves is of course variable with conditions. In these tests it usually ranged between 12 and 15 days. These results are therefore in accord with those obtained in the life history and seasonal development studies.

SUMMARY

An effort was made to devise a satisfactory apparatus for determining the spore content of orchard air. Preliminary experiments were made with an electrical device. The results were promising, but the greater economy and simplicity of a mechanical filter led to its adoption for this work. It is believed, however, that the electrical method has potentialities of adaptation to problems of this type.

By wetting leaves freshly collected from the orchard it was shown that, in the vicinity of Madison, Wis., asci were capable of discharge under favorable conditions on May 7, 1917. Because of dry weather, however, the first natural discharge was delayed until May 19. Following this date ascospore discharges occurred during rains throughout the spring and early summer, the last recorded discharge occurring on July 18. The very heavy discharges, however, were limited to the period May 19 to June 7. After this time the discharges were relatively small.

The maximal concentration of ascospores of *Venturia inaequalis* in the orchard air was observed on May 21, when the average rate of catch for a period of 8½ hours was 1 ascospore to 0.4 liters of air. The wind velocity for this period varied from 14 to 23 miles an hour. The filter was 3 feet above the ground.

It was found that, when the asci were in condition to eject their spores, the presence of an adequate supply of water was the most important requisite for their discharge. In the experiments dew was not sufficient to induce discharges of consequence.

In cases where abundant asci were in condition to eject their spores in the presence of water, heavy discharge started soon after rain began and continued with continuous rain as long as the supply of ripe asci lasted. These periods of very heavy discharge lasted from 3 to 15 hours. Undoubtedly the duration of such periods varies widely with conditions.

Conidia of *Venturia inaequalis* were found in the air only during rain periods, and particularly when rain was accompanied by strong wind. Experiments showed that these conidia are very resistant to detachment from their conidiophores when dry, but quickly become detached in the presence of water. They are, therefore, disseminated chiefly in meteoric water acting under the influence of wind and gravitation.

Germination tests showed that practically all naturally discharged ascospores were vigorously viable. Conidia germinated with much less regularity. At no time after their appearance in the spring, however, was it difficult to find viable conidia in abundance.

The seasonal development of the host plant was unusually slow in the spring of 1917. In the Turville orchard most of the blossoms were open by May 18, and petal-fall was about complete on May 25. The last leaves to develop appeared from about May 19 to 24, and very little leaf expansion occurred after June 8.

Bagging experiments and orchard observations showed that most of the leaf infection of the season occurred during the period of May 19 to June 2, during which time all but one of the major ascospore discharges of the season occurred. The first observation of leaf lesions was made on June 2, 14 days after the first period of ascospore discharge. As the leaves approached maturity they became highly resistant to infection by the scab fungus.

Fruit infections were observed on June 8 and continued to appear throughout the period of observation, which ended on August 24. The records indicate that the first fruit infection occurred during the rainy period of May 19 to 23.

Successful leaf inoculation experiments were conducted in the orchard upon branches inclosed in specially constructed moist chambers. Infection from conidia applied in suspension in water was secured at will upon young leaves if fairly low temperatures (maximum at which infection occurred somewhat above 25°C.) were maintained. Old leaves were highly resistant to infection by *Venturia inaequalis*.

RELATION BETWEEN MORTALITY OF TREES ATTACKED BY THE SPRUCE BUDWORM (CACOECIA FUMIFERANA CLEM.) AND PREVIOUS GROWTH [1]

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INTRODUCTION

In a recent bulletin [3] reference is made to the local variation in mortality following spruce budworm defoliation. Several factors are considered as influencing the percentage of mortality, namely: Character of defoliation, available soil moisture, effects of severe winters, maturity, composition of the forest, and vigor of the trees.

But little data bearing directly on this last factor had been collected. In an earlier paper [4] the following quotation occurs: "The dying may be likened to a greatly accelerated natural thinning that takes place in a normal forest. It is suppressed or overmature trees that die first; younger stands suffer least."

In the first quoted bulletin [5] the writer makes the following statement: "The vigor of the stand at the time of the budworm attack largely determines the condition in which it will come through." The higher mortality in older forests as compared to younger forests is very striking, yet often spotted and by no means uniform; in second-growth stands, which suffer less, areas of high mortality are frequently found.

In comparing the rate of growth of trees dying on sample plots it was found that the slow-growing trees died first as well as those receiving heaviest defoliation, as illustrated by balsam in Table I.

A more detailed study of this feature was contemplated for the summer of 1922, but time did not permit its fnl-

TABLE I.—*Radial increment in millimeters for 10-year period before budworm attack of trees dying at different periods*

[The first feeding occurred in 1918, the first mortality in 1920. Based on 2-acre plots containing over 350 trees, Lake Opasatika, Quebec]

	Summer 1920	Winter 1920–21	Summer 1921	Winter 1921–22	Summer 1922	Winter 1922–23
Increment in mm	8.2	10.5	10.9	11.7	14.0	15.4
Percentage of defoliation [a]	77	81	70	62	57	46
Average diameter, in inches	5.9	4.8	5.7	6.7	6.9	6.1

[a] Percentage of defoliation refers to old needles only at the end of the outbreak in 1921, or earlier in case of death. The new growth was completely destroyed each year from 1918 to 1921, inclusive.

[1] Received for publication April 22, 1924; issued June, 1925.

[2] This study was conducted by the writer as part of the 1923 program of work of the Division of Forest Insects, Dominion Entomological Branch, Ottawa, Canada.

The undertaking was greatly facilitated through the interested assistance of R. E. Balch and H. J. MacAloney, graduate students from Guelph College, Ontario, and the New York State College of Forestry, respectively. The collection of the data for Tables IV to IX and parts of Tables XII and XIII was made by the writer in person. On the remainder of the plots both the selection and tabulation were left entirely to the discretion of Balch and MacAloney. The compilation of the data was made by all three members of the crew. J. D. Tothill also assisted in taking some plots.

W. G. Wright, in charge of research work for the Dominion forestry branch, Ottawa, kindly assisted with suggestions concerning the tabulation of the data. E. N. Munns, Raphael Zon, and S. T. Dana offered valuable assistance through constructive suggestions on the preliminary manuscript.

This presentation represents a considerable condensation of the original manuscript, copies of which, with complete tables and data, are filed at the Bureau of Entomology and Forest Service, Washington, D. C., the Entomological Branch, Ottawa, and the New England Forest Service Experiment Station, Amherst, Mass.

[3] SWAINE, J. M., and CRAIGHEAD, F. C. STUDIES ON THE SPRUCE BUDWORM. Canada Dept. Agr. Bul. (n. s.) 37, 92 p., illus. 1924.

[4] CRAIGHEAD, F. C. BUDWORM INFESTATION VS. PULPWOOD PRODUCTION. 2. Amer. Paper and Pulp Assoc., Woodlands Sect. Ser. Proc., Ann. Meeting 2: 8–10, 1922.

[5] Footnote 3, Part II of this bulletin.

fillment. During the summer of 1923 data on a series of plots in various forest types were taken near Bathurst, New Brunswick, and Metis Lake, Quebec.

The studies were concentrated on second-growth forests. Mature forests, where the mortality was much higher, were avoided, owing to the complication of other factors, such as periodic cuttings. It was also deemed advisable to put more time on the second-growth stands, since such results would be more directly applicable to forest conditions of the future.

EXPLANATION OF DATA

In the softwood forests one-tenth acre plots were used. It was found that this size suited the purpose better, since the variation in injury was quite local and spotted. The percentages of balsam, red spruce, and white spruce, and the basal area and average diameter are based on the total of softwoods only; the percentage of hardwoods is that of the total number of trees on the plot. The average diameter was computed from the basal area. All trees from 3-inch diameter breasthigh and up were tallied and all data computed on this basis. This naturally gives a low expression for average diameter.

The percentage of mortality was computed on the basis of number of trees rather than on volume or basal area. No volume tables were available for these small diameters, and it was thought that percentages by trees would give a better expression of the effects of defoliation on the plots, especially the suppressed ones. In cases where the injury is low, it would often be quite negligible if computed in volume.

All increments are expressed in millimeters, showing the radius for a 10-year period unless otherwise stated. In each plot a varying number of cores were taken at breastheight (by means of an accretion borer), depending on the percentage of the species and number of each dead. At least 10 cores from dead and living trees of each species were secured, or, if the number of trees in such classes was small, at least half were taken. No definite method of selecting trees was used; the plot was gone through and all trees taken as encountered selected from all diameter classes. In computing the average rate of growth for each species on the plot, averages were first obtained from the selected cores for both dead and living trees. These averages were multiplied by the total number of dead and living trees, respectively, added and divided by the total number of that species on the plot. On the first few plots the average rate of growth for each species was determined from dominant trees only, average-diameter trees, all trees or the method described. The variation between different plots as expressed by dominant trees and average-diameter trees was considerable, but that between the last two methods was so slight that the one involving less time was adopted.

The 10-year period previous to budworm attack was counted back from the enlarged ring produced by the defoliation. This is very marked on balsam. In spruce, where the enlargement is less pronounced, the year of budworm attack (previously determined for the region) can be counted back from the last ring formed, but this is not reliable for balsam, owing to the fact that two or three rings may fail to form on parts of the circumference and not show on the increment core. On dead or dying trees, both spruce and balsam, the enlarged ring must be utilized for orientation, since from two to four rings may fail to form previous to death.

CHARACTER OF BUDWORM DEFOLIATION [6]

A brief description of the character of the budworm feeding will help to explain certain results of the tabulated data. Previous investigations have shown that the severity of defoliation is the primary cause of death of the trees through inhibiting normal physiological functions.

The degree of defoliation of the spruce and balsam is chiefly due to the variation in development of the new growth, to the migratory habits of the larvae, and to the fact that the old foliage of spruce is not consumed by the larvae. For normal development, the young caterpillars require new foliage as food. They begin feeding at the time the balsam and white-spruce buds open. During epidemics their abundance is such that the new growth of balsam is consumed by the time the caterpillars are half grown; in the later instars they are able to subsist on the old needles and consume up to 100 per cent of these. White spruce furnishes a greater abundance of new growth, so that rarely do the larvae consume all of it before the needles harden. This hardening of the needles takes place about the time the caterpillars are half grown, causing

[6] For more detail see reference, footnote 3.

them to migrate in search of more succulent food. It is possible that slower-growing white-spruce trees putting out a less quantity of new growth are entirely defoliated of the current year's needles before they harden. Red spruce, the buds of which open 10 to 12 days later than balsam and white spruce (shortly before the larvae begin migrating), furnishes a second supply of succulent needles at a time when the larvae are most voracious; consequently much of it is consumed.

The density of the stand affects the degree of defoliation in that migrating larvae have a better chance of falling on other food before striking the ground, from which they can not regain the trees. As a result of this migrating habit of the larvae no trees, even balsam, standing in the open are defoliated of more than their new growth.

Higher percentages of balsam and white spruce encourage to a certain extent heavier defoliation on account of the greater supply of desirable food (early new growth), which enables the larvae to develop rapidly in their early stages.

Balsam is always more severely defoliated than white or red spruce, since the old needles are also consumed; consequently, the feeding is less uniform and offers an explanation for lack of correlation between mortality and rate of growth under conditions of severe feeding. White and red spruce are defoliated only of the new growth, white usually to a lesser degree than red, and both more uniformly than balsam. The amount of defoliation on the balsam sample plots at Lake Opasatika, Quebec, from 1918 to 1921 is shown in Table II.

Dominant trees, especially those highest in the stand, are less severely defoliated than those beneath, owing to the migrating habits of the larvae. Table III illustrates this feature on the balsam sample plots at Lake Opasatika. The figures apply only to old foliage, since all the new growth was consumed each year.

Thus the understory, which at the same time is composed of slower-growing trees, receives more defoliation. This does not apply to light outbreaks when, with abundance of food, the larvae do not migrate.

SECOND-GROWTH SOFTWOOD AND BUDWORM MORTALITY

Two widely separated areas were considered in these studies, centering about Bathurst, New Brunswick, and Metis Lake, Quebec.

The Bathurst plots were located about 15 to 20 miles south of Bathurst, on the Tabusintac drainage. The soil is of a light sandy character, formed from the millstone grit of the middle Carboniferous. It is fairly thin and subject to excessive drying out, except on the hardwood ridges, where it is much deeper and of a loamy character. It fairly well characterizes the Miramichi watershed, noted for its spruce forests.

The other series of plots were taken on the south shore of the St. Lawrence River near the height of land between the Metis and Patapedia Rivers, on a seigniory of Price Brothers at Metis Lake, Quebec. It is essentially a softwood region, only scattered yellow and white birch occurring, except on the tops of the higher hills. Some 40 years ago the area was very heavily cut over, so that the present stand averages about 75 years of age. A few older trees occurred on many plots. An attempt was made to secure the plots in more uniform younger growth and at the same time to select plots which showed higher mortality. Consequently, these figures are not quite typical of the area.

In the vicinity of Bathurst 40 plots were tallied in practically pure softwood stands. An attempt was made to group these by series conforming to site qualities, types, and age classes. Tabulation of 24 plots is given occurring on a 65-year burn representing three sites of the spruce flat type. The remaining plots were more scattered in

TABLE II.—*Percentage of trees in arbitrary defoliation classes*

Degree of defoliation	100	90	75	50	25	10
Percentage of trees in class	5.6	10.6	16.9	33.2	23.8	9.9

TABLE III.—*Percentage of defoliation by diameter classes*

Diameter breasthigh, inches	2	3	4	5	6	7	8	9	10	11	12
Number of trees	4	47	65	31	57	54	44	30	19	11	11
Average defoliation, per cent	54	77	66	64	51	46	42	29	28	28	14

various age classes up to 100 years and in jack pine and spruce swamp types. In general they conformed to the results here shown, but the number of plots on each condition was not sufficient to be conclusive, and consequently they are not included. Altogether, measurements were recorded from over 3,000 increment cores.

The defoliation on these plots was very severe, and all evidence indicates that it was quite uniform for the entire area.

The Metis plots, Tables X and XI, were chosen because of light budworm feeding for comparison with the heavy feeding in the Tabusintac area. The attack began in 1913. Although the percentage of balsam was high, the feeding apparently only lasted, with any degree of severity, for one year. This was reported from observations of the company officers who frequently visit the place during the summer. It is also further substantiated by the more rapid recovery, or greater increment for the 10-year period following defoliation, as compared to any other regions of Quebec and New Brunswick which have been studied. (See Table XVIII.)

The reason [7] for the light feeding, which is certainly due to the early dying out of the infestation, has been unexplained. It may have resulted from weather conditions. A similar state prevails eastward throughout the Gaspe Peninsula.

This series of plots was selected and tallied by Balch and MacAloney. The writer had previously visited the region on two occasions, but did not go over the present work. No attempt was made to segregate these plots by types or quality sites.

This region as a whole is characterized by preponderance of white spruce over red spruce.

TABLE IV.—*Composition of plots, Bathurst, New Brunswick*

No.	Balsam	Red spruce	White spruce	Hardwoods	Number of trees	Average diameter [a]	Basal area	Height	Largest trees [b]	
									Number	D. b. h.
	Per cent	*Per cent*	*Per cent*	*Per cent*		*Inches*	*Sq. in.*	*Feet*		*Inches*
7a	7.1	92.9	0	0	70	5.3	10.68	45	6	8
7	0.0	100.0	0	0	108	5.7	19.02	55	3	9
8	3.8	96.2	0	0	105	5.6	18.40	50	2	10
9	2.1	97.9	0	0	96	5.7	17.21	55	3	9
7c	13.2	86.8	0	0	106	5.9	20.31	60	2	10

[a] Includes trees 3 inches d. b. h. and over.

[b] The expression "Largest trees, d. b. h.," indicates the diameter class of the largest trees, and "Number," the number of trees in that class.

TABLE V.—*Radial increment in millimeters at d. b. h. from 1903 to 1912, inclusive, and mortality, of trees in plots of Table IV* [a]

No.	Dead spruce	Radial increment of spruce		
		Living	Dead	All
	Per cent			
7a	13.8	8.6	5.8	8.2
7	14.7	8.4	7.3	8.2
8	21.7	7.3	5.8	7.0
9	50.0	6.7	6.2	6.4
7c	49.0	5.5	4.4	5.0
Average		7.6 (94)	5.8 (54)	6.9

[a] Figures in parentheses refer to number of measurements.

[7] Heavy rainstorms at the time of opening of the buds and severe frosts, killing the new growth, have both been reported by Tothill and Craighead as causing high mortality in the budworm larvae.

TABLE VI.—*Composition of plots, Bathurst, New Brunswick*

No.	Balsam	Red spruce	White spruce	Hardwoods	Number of trees	Average diameter	Basal area	Height	Largest trees	
									Number	D. b. h.
	Per cent	*Per cent*	*Per cent*	*Per cent*		*Inches*	*Sq. in.*	*Feet*		*Inches*
3d	12.6	67.2	21.2	10.7	67	6.8	16.75	60	5	11
3c	4.8	75.0	20.2	1.2	84	6.8	20.49	60	2	11
3b	7.7	62.6	29.7	1.0	91	7.1	24.87	60	2	14
3a	30.2	52.4	17.4	1.5	63	8.0	24.17	70	1	15
9b	8.2	91.8	0	15.5	98	6.0	19.49	57	3	10
9a	5.8	94.2	0	8.4	87	6.1	17.77	55	2	10
6a	5.5	67.5	27.0	0	111	6.3	24.35	56	2	11
6b	1.2	67.4	31.4	11.9	89	6.1	18.20	56	3	10

TABLE VII.—*Radial increment in millimeters at d. b. h. from 1903 to 1912, inclusive, and mortality of trees in plots of Table VI* [a]

No.	Balsam, increment				Red spruce, increment				White spruce, increment				All spruce	
	Dead	Living	Dead	All	Dead	Living	Dead	All	Dead	Living	Dead	All	Dead	Increment
	P. ct.	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*
3d	14.3	9.7	------	9.7	2.2	10.6	------	10.6	7.1	13.3	------	13.3	3.4	11.2
3c	50.0	7.0	------	7.0	3.2	10.6	6.0	10.4	35.3	11.4	4.6	9.0	10.0	10.3
3b	28.5	12.0	3.0	9.4	10.8	9.9	------	9.9	44.4	10.0	5.9	8.1	15.4	9.3
3a	100.0	------	9.2	9.2	15.1	9.4	7.2	9.1	36.4	8.9	6.0	7.8	20.5	8.8
9b	50.0	16.0	17.0	16.5	23.3	9.0	7.2	8.6	------	------	------	------	23.3	8.6
9a	100.0	------	7.7	7.7	40.3	9.0	6.2	7.8	------	------	------	------	40.3	7.8
6a	33.3	------	------	------	40.0	8.9	7.0	8.1	66.6	8.6	4.9	6.1	47.6	7.6
6b	100.0	------	------	------	41.7	8.8	5.5	7.4	53.7	10.0	2.7	6.1	45.4	7.0
Average	------	11.6 (12)	9.4 (16)	10.4	------	9.5 (118)	6.5 (54)	8.5	------	10.5 (40)	4.8 (29)	8.1	------	------

[a] Figures in parentheses refer to number of measurements.

TABLE VIII.—*Composition of plots, Bathurst, New Brunswick*

Plot No.	Balsam	Red spruce	White spruce	Hardwood	Number of trees	Average diameter	Basal area	Height	Largest trees (65 years)	
									Number	D. b. h.
	Per cent	*Per cent*	*Per cent*	*Per cent*		*Inches*	*Sq. in.*	*Feet*		*Inches*
18	21.4	28.6	50.0	0	57	6.9	15.42	70	2	15
7b	53.2	42.0	4.8	26.2	62	6.0	12.78	65	1	12
17a	1.2	73.2	25.6	0	82	7.0	22.11	65	2	12
16	11.6	26.8	61.6	0	112	6.5	25.61	65	3	11
17	22.2	29.6	48.2	0	54	8.4	20.75	68	3	12
15	15.6	31.1	53.3	2	90	7.9	26.32	65	3	10
7	30.3	63.2	6.5	10.6	76	6.2	15.96	60	2	10
7a	39.5	51.1	9.4	2.3	86	6.5	19.97	61	2	11
14a	26.4	46.1	26.4	4.7	121	5.8	22.37	60	6	9
13	15.6	6.9	77.4	0	116	7.0	31.08	60	1	13
14	57.6	5.6	36.8	0	126	5.8	23.46	60	1	10

TABLE IX.—*Radial increment in millimeters in d. b. h. from 1903 to 1912, inclusive, and mortality in trees of plots of Table VIII* [a]

Plot No.	Balsam, increment				Red spruce, increment				White spruce, increment				All spruce	
	Dead	Living	Dead	All	Dead	Living	Dead	All	Dead	Living	Dead	All	Dead	Increment, all
	P. ct.	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*
18	0	15.1	------	15.1	0	9.4	------	9.4	0	9.7	------	9.7	0	9.6
7b	64.2	11.7	7.8	8.9	3.8	9.1	13.0	9.3	33.3	12.7	2.0	9.8	4.8	9.2
17a	0	------	------	------	5.0	11.9	13.3	11.9	14.3	6.2	4.5	6.0	7.4	8.0
16	77.0	------	10.6	10.6	16.6	10.6	7.8	10.1	29.0	7.1	3.1	5.9	25.2	7.2
17	91.7	------	9.1	9.1	43.7	6.3	7.1	6.0	30.8	8.1	4.6	7.0	35.7	6.9
15	100.0	------	6.1	6.1	28.6	7.0	7.2	7.1	45.8	9.2	4.8	7.2	39.5	7.1
7	91.4	------	7.0	7.0	40.0	7.2	7.6	7.3	60.0	5.0	2.0	3.3	41.5	7.0
7a	97.1	------	9.7	9.7	52.3	6.9	6.6	6.7	50.0	5.0	2.5	3.7	52.0	6.3
14a	100.0	------	6.2	6.2	50.9	7.6	6.3	6.9	65.6	5.0	3.4	4.0	56.2	6.0
13	61.1	------	7.7	7.7	50.0	10.7	8.5	9.6	75.3	6.8	4.3	4.9	73.2	5.3
14	100.0	------	6.2	6.2	71.4	------	------	------	80.4	6.6	5.3	5.4	79.2	5.4
Average	------	13.5 (11)	7.4 (68)	8.3	------	8.9 (91)	7.5 (55)	8.3	------	7.5 (68)	4.2 (61)	5.9	------	------

[a] Figures in parentheses refer to number of measurements.

TABLE X.—*Composition of plots, Metis Lake, Quebec* [a]

Balsam	Dead balsam	Red spruce	Dead red spruce	White spruce	Dead white spruce	Hardwoods	Average number trees per plot	Average diameter	Average basal area	Average height	Average age, in years
Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*		*Inches*	*Sq. in.*	*Feet*	
79.1	39.1	5.3	0	15.6	11.6	5.7	88.8	6.7	21.14	60	70

[a] Based on 33 plots.

TABLE XI.—*Radial increment in millimeters from 1903 to 1912, inclusive, and mortality of trees in plots of Table X. Plots averaged by groups in percentage classes* [a]

Dead	Balsam, increment			White spruce, increment			Red spruce, [b] increment
	Living	Dead	All	Living	Dead	All	Living
Per cent	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
0 to 5	12.4	2.0	12.3	11.8	3.2	11.1	----------
5 to 15	10.6	5.0	10.4	10.6	5.3	10.0	----------
15 to 25	10.2	5.0	9.2	11.2	5.8	10.0	----------
25 to 35	9.5	5.3	8.0	9.3	3.5	7.6	----------
35 to 45	8.3	5.0	6.9	----------	----------	----------	----------
45 to 55	8.8	4.4	6.7	7.5	2.5	5.0	----------
55 to 65	8.1	5.2	6.3	7.5	3.5	5.1	----------
65 to 75	7.8	4.4	5.2	----------	----------	----------	----------
Average	9.0 (507)	5.0 (335)	7.4	9.6 (220)	4.3 (43)	8.8	8.4 (77)

[a] Figures in parentheses refer to number of measurements.
[b] Too few trees per plot to be considered.

From the data collected and from general observations made, certain conclusions are drawn concerning budworm mortality and silvicultural characteristics of balsam fir, white spruce, and red spruce. These, since they are based on limited areas, are presented as tentative and with the object of inviting criticism and further investigations from the foresters and entomologists familiar with the budworm infested regions. It is fully realized that with so many factors to consider it is practically impossible to make comparisons of conditions alike except for one factor and that the results brought out in this paper are largely suggestive rather than conclusive.

There is a certain correlation between vigor of the stands (as expressed by increment at breastheight) at the time of defoliation and budworm mortality such that it can be said the more rapid the rate of growth the lower is the mortality resulting from defoliation. This expression of the probable effects of budworm feeding is a more tangible quantity than any of the other factors heretofore considered, and should serve as a practical basis for preventive measures through proper silvicultural practices which maintain rapid growth.

There may be different minimum rates of growth, entailing immunity for different sites, types, and age classes, indicating the necessity of further investigation in conditions other than those discussed here.

All the data bearing on density and basal area were plotted with the assistance of E. N. Munns, but no correlation was found. Percentage of balsam in the mixture was likewise considered, but this bore no relation to the mortality of the spruce.

The correlation between rate of growth and mortality for balsam is not at all regular for the Bathurst series (figs. 1 and 2, Tables IV to IX), owing to the severe infestation in that region, resulting in complete defoliation of many trees. From 75 to 100 per cent defoliation will kill even very vigorous trees. The correlation for the white and red spruces is much more regular. These trees are more uniformly defoliated, only the new growth being eaten for three to four years; that for combined red and white spruce is still more regular, the explanation being doubtful unless it is because of the greater number of trees averaged. On the Metis area (Tables X and XI, fig. 2) the correlation between rate of growth and mortality for balsam is quite regular; that for white spruce shows very little correlation. Here the dead spruce trees are all small suppressed trees of the understory (see Table XVII, mortality by diameter classes) as compared with the Bathurst spruce and Metis balsam. In fact, so few spruces are dead that the entire mortality in these trees is considered to be that of a normal forest and gives a series of plots for contrast to conditions at Bathurst.

The diameter class tallies by species as well as by observation on the stumps of many felled trees indicate that

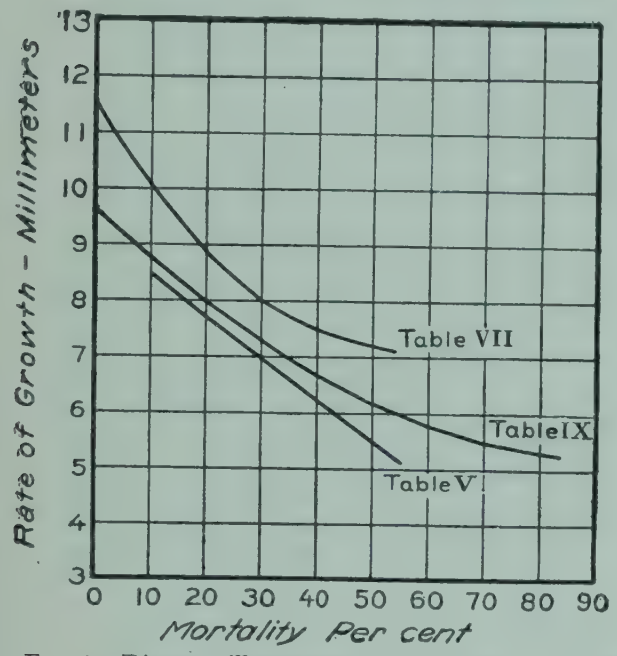

FIG. 1.—Diagram illustrating rate of growth of spruce and mortality resulting from spruce budworm defoliation as recorded in Tables V, VII, and IX

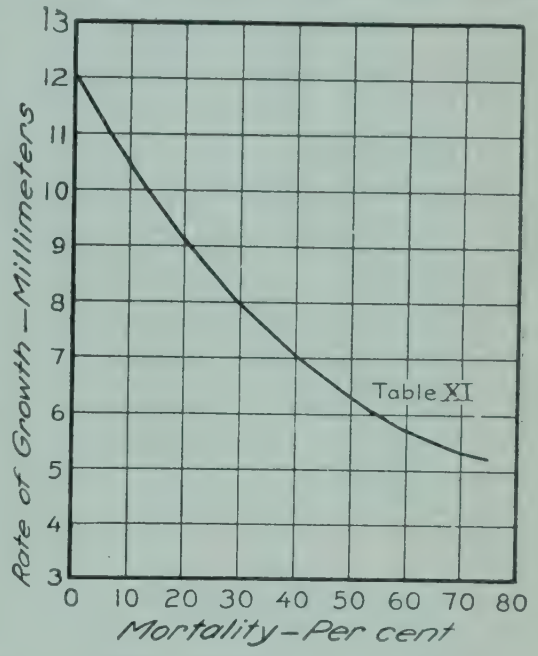

FIG. 2.—Diagram illustrating rate of growth of fir and mortality resulting from spruce budworm defoliation as recorded in Table XI

second-growth white spruce grows faster than either red spruce or balsam during the first 40 to 50 years, but after certain densities are reached its growth is retarded and it can not compete as well as red spruce. Considering the higher mortality of white spruce compared to that of red spruce in 60-year stands, the relatively lighter feeding (excepting the possibility that slow-growing trees may be subject to heavier defoliation) and the average slower rate of growth for dying trees, it may be considered that white spruce is more susceptible to budworm attacks than red spruce. This suggests that it should be encouraged only when good vigor can be maintained. Also a higher percentage of white spruce, like balsam, enhances heavier average defoliation of adjacent red spruce owing to the abundant food supply in the early feeding stages.

Balsam is the most susceptible under all conditions, since it is completely defoliated in heavy outbreaks. On the other hand, the Metis plots indicate, as well as certain Bathurst plots, that vigorous stands are more immune. Since balsam is a very fast grower, and reproduces abundantly and under adverse conditions, it might well be encouraged for the first 30 to 40 years. The only argument against it is that over wide areas it is considered the most important factor in giving impetus to an outbreak, though balsam is probably no more effective in this respect than is a mixture of white spruce and red spruce.

HARDWOOD MIXTURES AND BUDWORM MORTALITY

Two series of plots (Tables XII, XIII, XIV, and XV) were taken in hardwood mixtures. The object was to obtain some idea of the effect of budworm feeding on the free and overtopped softwoods in such stands. It has been held by several investigators that the mortality in hardwood mixtures is always considerably lower than in pure softwoods.

Two types were considered. Tables XII and XIII summarize 19 plots, totaling 3 acres, in a 60 to 65 year birch-poplar type on the same area as the softwood plots of Tables IV, VI, and VIII. Tables XIV and XV summarize 11 plots, totaling 3½ acres, in a northern hardwood type on the Bathurst area. This area has been subjected to periodic cuttings in the past. No white spruce occurred. The largest diameter for softwoods was about 20 inches. These plots were located on the top of a low ridge on the best growing site in the region.

Percentage expressions are the same as for previous plots except that total percentage of hardwoods is calculated on the basis of all trees on the plots. This expression was not considered a fair indication of the hardwood canopy, so the percentage of overtopped softwoods is used as indicating the amount of hardwood canopy. Two classes of softwoods were considered—overtopped, those whose terminals were under the softwood canopy, and free, those

TABLE XII.—*Birch and poplar type, composition of plots*

Balsam	Balsam, overtopped	Red spruce	Red spruce, overtopped	White spruce	White spruce, overtopped	Softwoods, overtopped	White birch	Poplar	Total hardwoods	Average number trees per 1/10 acre	Softwood, average diameter	Basal area, 1/10 acre
P. ct. 34.2	*P. ct.* 39.2	*P. ct.* 50.3	*P. ct.* 38.6	*P. ct.* 15.4	*P. ct.* 32.0	*P. ct.* 60.7	*P. ct.* 70.7	*P. ct.* 29.3	*P. ct.* 24.2	70	*In.* 6.3	*Sq. ft.* 15.34

TABLE XIII.—*Radial increment in millimeters at d. b. h. from 1903 to 1912, inclusive, and mortality of trees in plots of Table XII*

	Balsam, increment				Red spruce, increment				White spruce, increment			
	Dead	Living	Dead	All	Dead	Living	Dead	All	Dead	Living	Dead	All
	P. ct.	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*	*Mm.*	*Mm.*	*P. ct.*	*Mm.*	*Mm.*	*Mm.*
Free	78.5	17.1	10.9	11.9	23.6	10.8	10.7	10 8	18.7	11.9	6.4	10.9
Overtopped	38.8	6.8	6.6	6.7	7.6	8.7	7.4	8.6	21.8	8.9	6.4	8.4
Average	228 trees			8.2	293 trees			9.5	117 trees			9.7

TABLE XIV.—*Northern hardwood type, composition of plots*

Balsam	Balsam, overtopped	Red spruce	Red spruce, overtopped	White spruce	White spruce, overtopped	Softwoods, overtopped	Yellow birch	Maple	Beech	Hemlock	Total hardwoods	Number trees, 1/10 acre	Softwood, average diameter	Basal area, 1/10 acre
P. ct. 46.7	*P. ct.* 38.8	*P. ct.* 53.3	*P. ct.* 35.7	*P. ct.* 0	*P. ct.* -------	*P. ct.* 59.7	*P. ct.* 20	*P. ct.* 15.3	*P. ct.* 64.7	*P. ct.* 0.4	*P. ct.* 27.4	32.2	*In.* 6.8	*Sq. ft.* 7.20

TABLE XV.—*Radial increment in millimeters at d. b. h. from 1903 to 1912, inclusive, and mortality of trees in plots of Table XIV*

	Balsam, increment				Red spruce, increment			
	Dead	Living	Dead	All	Dead	Living	Dead	All
	Per cent	*Mm.*	*Mm.*	*Mm.*	*Per cent*	*Mm.*	*Mm.*	*Mm.*
Free	58.8	17.7	14.9	16.3	39.6	18.4	22.1	19.8
Overtopped	9.9	10.7	7.5	10.1	5.4	10.0	5.5	8.2
Average	206 trees			13.2	234 trees			14.6

whose terminals were above the canopy or standing in an opening in the hardwood foliage. Since no comparison of individual plots was contemplated, the tabulated data are a summary for all plots.

The mortality of four softwood series at Bathurst and Metis (Tables IV to XI) and two hardwood series (Tables XII to XV) is compared in Table XVI. The hardwood mixtures show considerably less mortality for white spruce and red spruce, though this is not so marked for balsam. If the percentages of mortality among the free and overtopped trees are compared, it is seen that the reduction in mortality is chiefly due to the overtopped trees.

In these series of plots the rate of growth for the overtopped trees is considerably lower than for free trees, so this factor would tend to increase mortality if it were not overbalanced by the protection offered by the hardwood canopy. The average rate of growth for the species of softwood trees in the hardwood series (Tables XII, XIII, XIV, and XV) is lower for balsam and higher for both red and white spruce than in the preceding softwood series.

In the northern hardwood type (Tables XIV and XV) the average rate of growth for all balsam (13.2) and red spruce (14.6) is considerably higher than for any other series of plots. Likewise the mortality of the overtopped softwoods is lower and shows a greater contrast to the free dead softwoods. The total mortality for balsam is lower than in the birch-poplar type (Tables XII and XIII), while that for red spruce is higher. It so happens that in these plots the rate of growth for the dead red spruce is higher than for the living. This discrepancy may be due to the method of tabulating free and overtopped trees, which does not properly group the trees according to the relative amount of defoliation. Balch recognized this, adding the following note on one plot:

> The free living red spruce are smaller than the free dead red spruce. The free living are in many cases partly overtopped, the free dead generally almost entirely free. Thus the percentage of canopy seems a greater factor than rate of growth.

These free living dead trees are thus subjected to a greater foliage exposure, and consequently to heavier defoliation, than those which survived and are partly overtopped, though classed as free because the terminal was free.

The lower percentage of mortality of the softwoods in hardwood mixtures is entirely a matter of protection by the hardwood foliage. This protective effect is due to concealment of overtopped softwoods from the ovipositing moths and the lessened chance of migrating larvae falling on the softwoods.

The mortality among the free softwoods of hardwood mixtures is about the same or slightly higher for balsam than for the pure softwood. This substantiated many observations in the field where high mortality was observed, especially in older hardwood mixtures when the hardwoods were

becoming decadent, and suggests that the conifers, after years of competition in hardwood mixtures, are even less resistant than those in softwood stands.

Some indication of the relative ability of these trees to withstand severe competition is obtained from the tables summarizing the hardwood plots (Tables XII, XIII, XIV, and XV) and that of the mortality by diameter classes (Table XVII), suggesting that white spruce is least resistant as indicated by the higher mortality of the overtopped trees of the birch-poplar type (Tables XII and XIII), and the higher percentage of mortality in the 3, 4, and 5 inch diameter classes of softwood types. The same tables indicate that balsam can withstand more competition than white spruce and that red spruce is the most resistant species.

MORTALITY BY DIAMETER CLASSES WITH SEVERE AND LIGHT DEFOLIATION

The following table of the tree mortality (Table XVII) of three series of plots tabulated by diameter classes is given to show the relative effects of different degrees of caterpillar feeding on the various softwoods concerned and how such feeding affects mortality in the larger and smaller tree classes.

decreasing in the smaller and larger trees; in white spruce highest mortality occurs in the smaller-diameter classes, decreasing in the higher diameters.

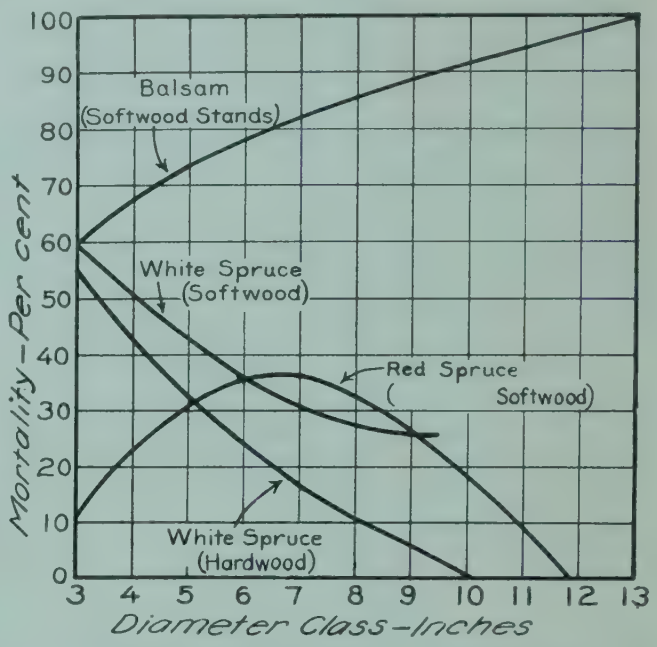

FIG. 3.—Mortality of spruce and fir in various forest types resulting from severe defoliation by the spruce budworm, shown by diameter classes. The white spruce (hardwood) are free

Considering the same combination but with lighter feeding (fig. 4) as occurred at Metis, where the red and white spruce are very little affected (in fact, it might be questioned if

TABLE XVI.—*Percentages showing softwood composition before budworm attack and total mortality for each series of plots*

	Balsam		Red spruce		White spruce	
	Mortality	Composition	Mortality	Composition	Mortality	Composition
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Softwoods from Tables IV and V	5.2	66.2	94.8	29.8		
Softwoods from Tables VI and VII	9.5	59.5	72.1	22.1	18.4	40.6
Softwoods from Tables VIII and IX	27.4	60.2	33.3	27.9	39.2	37.3
Softwoods from Tables X and XI	79.1	39.1	5.3	5.5	15.6	11.6
Hardwoods from Tables XII and XIII	34.2	53.0	50.3	13.4	15.4	20.2
Free trees (alone)	60.8	78.5	61.3	23.6	68.0	18.7
Overtopped trees (alone)	39.2	38.8	38.6	7.6	32.0	21.8
Hardwoods from Tables XIV and XV	46.7	27.2	53.3	20.5		
Free trees (alone)	61.2	58.8	64.3	39.6		
Overtopped trees (alone)	38.8	9.9	35.7	5.4		

Considering the mortality of the three species of trees concerned by diameter classes and under conditions of heavy defoliation (fig. 3) (such as is represented by softwood stands of Tables VIII and IX) it is shown that in balsam mortality is relatively high in all classes, but increases with diameter; in red spruce highest mortality occurs in the middle-diameter classes, mortality in these two species can be attributed to other than natural thinning), we find that in balsam greatest mortality occurs in smaller-diameter classes, decreasing with larger diameters; in red spruce all mortality occurs in low-diameter classes below 6 inches; in white spruce all mortality occurs in low-diameter classes below 6 inches.

Considering mortality in the hardwood plots, Tables XII and XIII, where defoliation was severe on the free trees and relatively lighter in the overtopped trees, it is shown that balsam mortality of free trees is similar to that in softwoods (as illustrated in fig. 3); among the overtopped trees it falls quite regularly from the smaller diameters to the higher. Red spruce mortality among the free trees is similar to that of softwood stands (as illustrated in fig. 3); among the overtopped trees it falls quite regularly from the smaller diameters to the higher. White spruce mortality among the free trees falls rapidly as diameter increases and is lower than white spruce in the softwoods; among the overtopped trees the mortality falls similarly to that of red spruce, although it is higher in all diameters.

It might be assumed that the correlation drawn between rate of growth and mortality is not conclusive from the fact that the position of the tree in relation to the crown influences the amount of defoliation (intermediate and suppressed trees receive relatively more defoliation in severe epidemics), and degree of defoliation is no doubt the most important factor causing death. In other words, may not the higher mortality of slower-growing trees be a result of their position?

This is clearly not the case with balsam, which is subject to heavy defoliation, since greater mortality occurs as diameter increases, and there was found to be no correlation between rate of growth and mortality for such heavily defoliated balsam.

With red spruce receiving severe defoliation both in pure softwoods and free trees in hardwood stands the greatest mortality occurs between the diameter classes 6 and 8 inches, which trees in these 60-year stands would be largely either dominants or codominants.

In the case of white spruce receiving heavy defoliation, greatest mortality does occur in the lower-diameter classes 3, 4, and 5 inches, and here it is impossible to decide how much of the mortality is due to position, suppression, or budworm defoliation. In hardwood stands (Table XVII) greater total mortality occurs in the overtopped trees, which receive less defoliation, than in the free trees. On the other hand, with lighter feeding, mortality in all species is greater in the lower-diameter classes, which, of course, on the whole are slower-growing trees, but here again in balsam, which receives more defoliation, the mortality persists well up into high-diameter classes (both in softwood stands and overtopped trees in mixed hardwood stands) where many of the trees in softwood stands would be dominants and codominants.

It is not intended to argue that position does not have a certain effect on mortality, yet it seems impossible this long after the budworm feeding to determine just what weight can be attributed to it.[8] Referring to Table I, it will be seen that there is little difference in the average diameter of trees dying over a three-year period, while rate of growth and percentage of defoliation show regularly increasing or decreasing values in respect to time of death.

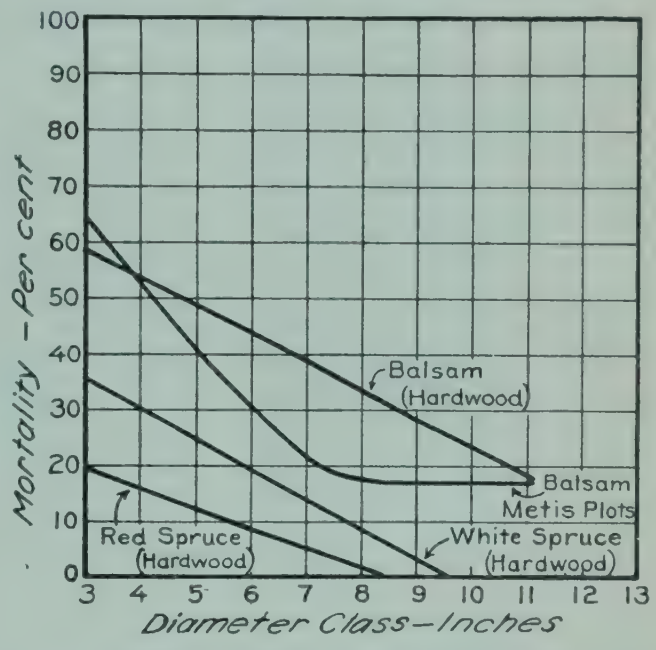

FIG. 4.—Mortality of spruce and fir in various forest types resulting from light defoliation in hardwood mixtures by the spruce budworm, shown by diameter classes. The balsam (hard wood), red spruce, and white spruce are overtopped

COMPARISON OF RADIAL INCREMENT OF SOFTWOODS BEFORE AND AFTER BUDWORM ATTACK

These series of measurements (Table XVIII and fig. 5) were taken to compare the rate of growth of partially defoliated trees before and after the budworm attack. The trees were selected promiscuously over the areas on which the foregoing plots were taken. Only dominant or codominant trees

[8] The degree of feeding on individual trees could not be determined at the time of this study.

TABLE XVII.—*Mortality by diameter classes (in percentages)*

SEVERE FEEDING

Tree species and association	3	4	5	6	7	8	9	10	11	12	13
Balsam:											
Softwoods of Tables VIII and IX	40	71	75	83	79	96	83	67	0	100	0
Free trees in hardswoods, Tables XII and XIII	50	62	82	79	70	93	79	92	90	83	100
Red spruce:											
Softwoods of Tables VIII and IX	19	9	36	39	33	32	29	7	11	0	0
Free trees in hardwoods, Tables XII and XIII	20	31	23	22	36	26	20	14	0	0	0
White spruce:											
Softwoods of Tables VIII and IX	48	60	56	12	32	32	21	32	21	0	0
Free trees in hardwoods, Tables XII and XIII	------	54	24	30	17	13	0	0	0	0	0

LIGHTER DEGREES OF FEEDING

Balsam:											
Softwoods of Tables X and XI	55	71	48	33	19	21	16	21	18	0	0
Overtopped trees in hardwoods, Tables XII and XIII	30	37	49	44	42	30	18	29	------	------	------
Red Spruce:											
Softwoods of Tables X and XI	0	25	3	0	0	0	0	0	0	0	0
Overtopped trees in hardwoods, Tables XII and XIII	57	11	8	9	12	8	0	0	------	------	------
White spruce:											
Softwoods of Tables X and XI	39	35	24	3	0	0	0	0	0	0	0
Overtopped trees in hardwoods of Tables XII and XIII	33	29	33	17	18	8	0	0	0	------	------

TABLE XVIII.—*Rate of growth before and after budworm attack*

FROM SPRUCE FLAT TYPE, BATHURST, NEW BRUNSWICK

	Balsam				Red spruce				White spruce			
	10 years before	5 years before	10 years after	Number of trees	10 years before	5 years before	10 years after	Number of trees	10 years before	5 years before	10 years after	Number of trees
	Mm.	*Mm.*	*Mm.*		*Mm.*	*Mm.*	*Mm.*		*Mm.*	*Mm.*	*Mm.*	
Living [a]	19.9	9.2	11.0	85	12.2	5.7	7.2	88	12.3	5.6	6.5	89
Dead [b]	12.2	5.4	------	199	9.3	4.4	------	192	5.5	2.2	------	133

FROM NORTHERN HARDWOOD TYPE, BATHURST, NEW BRUNSWICK

Living	18.9	10.4	12.5	90	19.8	10.0	13.0	116	------	------	------	------
Dead	18.7	9.9	------	63	16.6	9.6	------	76	------	------	------	------

FROM METIS LAKE, QUEBEC

Living	11.5	5.3	7.8	16	------	------	------	------	------	------	------	------

[a] From trees which recovered.
[b] From trees which died in 1922 from effects of defoliation

were bored. For this reason they can hardly be used to express the rate of growth of the regions as a whole so well as can the plot increments.

On the softwood plots the rate of growth for the 10-year period following first feeding (i. e., including 4-year feeding period and 6 years' recovery) was only about one-half that of the previous 10 years, while in the northern hardwood type this increment was about two-thirds the previous 10-year period. This may be due to greater ability of the trees to recover in the latter type, though possibly the trees scattered through these hardwoods were not so severely defoliated. These figures, as do previous tabulations, show that the white spruce dying from defoliation was growing at a slower rate than the red spruce which died.

The Metis Lake plots (Tables X and XI) unfortunately (due to misunderstanding) were based on too few trees to make fair comparisons possible.

SUGGESTED APPLICATION

The recent widespread series of budworm epidemics in eastern Canada and northeastern United States, coming as they did at a time when the softwood supplies of these regions are becoming depleted and when considerable interest in putting the forests on a sustained-yield basis is being manifested, will no doubt stimulate more intensive forest practices. At all events, the budworm is an ever-present menace to the growing of spruce and fir in these regions and must be given due consideration in the application of any silvicultural systems.

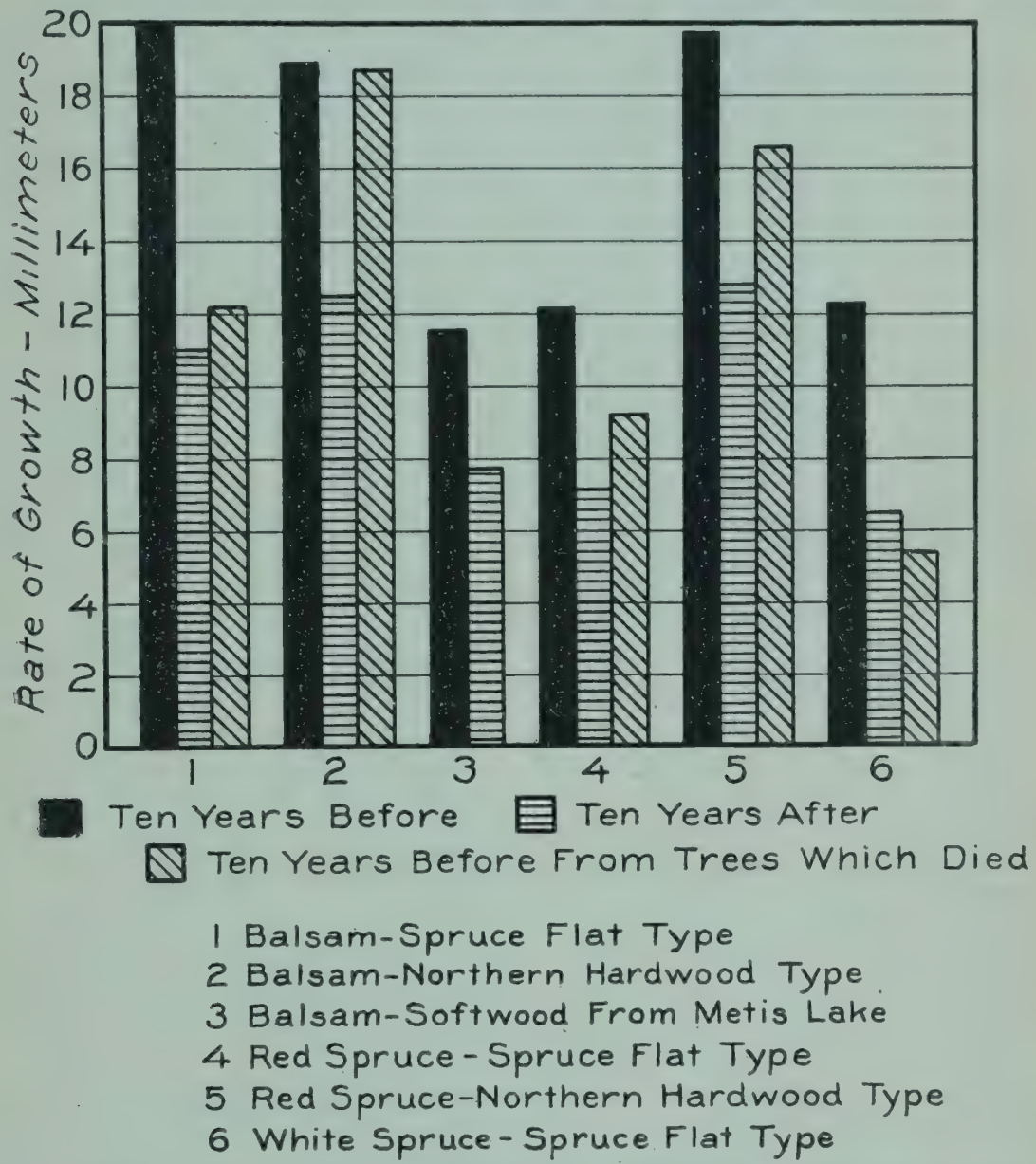

FIG. 5.—Diagram showing rate of growth of spruce and fir, occurring in various forest types, for the 10-year periods preceding and following spruce budworm defoliation

Our knowledge of how budworm outbreaks originate and the factors necessary for their phenomenal increase and spread is so meager that any recommendations for the prevention of future outbreaks are only speculative. When outbreaks do occur, we can hope for little relief from direct control measures, though prompt salvage of the defoliated material will greatly reduce the total losses.

With such limited possibilities of prevention or control before us, the only alternative is to keep the future forests in a condition least susceptible to the effects of defoliation. It is believed that the present study indicates that this can be accomplished by maintaining thrifty and vigorous stands.

This result can only be secured in natural stands by judicious cuttings to reduce the density and promote more rapid increment in the individual trees. As an example, in second-growth stands, such as those under consideration, varying from 20 to 50 cords per acre at 40 to 50 years of age, it would certainly be practical to make a pulpwood cutting when competition becomes so severe as to induce high mortality from defoliation. Such an operation should remove from 5 to 10 cords per acre, so as to make it profitable, and it should be conducted with the idea of maintaining trees of better quality.

The selection of the trees for thinning should be governed by the following considerations:

Remove all the balsam possible to the smallest possible diameter limit, since it is most susceptible to budworm defoliation, promotes heavy feeding, and may be an important factor in originating outbreaks. Any balsam that is left should be single, thrifty, dominant trees. Groups of balsam should never be left.

Remove all inferior red and white spruce and those that will not gain dominance by the time the next logging operation is contemplated.

Between doubtful red spruce and white spruce favor red spruce, since it better withstands adverse conditions and is a more persistent grower. The presence of red spruce is least effective in promoting severe budworm defoliation.

These thinnings should induce reproduction and, judging by the effect of bud-worm thinnings, this regeneration may be largely balsam. However, by removing practically all of the balsam and by breaking the soil litter through logging, possibly a high percentage of spruce can be secured.

If the stands are being managed for pulp wood, 10 to 20 years later, after reproduction is established, clear cutting should be adopted. In this case the new crop could again be mixed with balsam.

If saw material is desired later, pulpwood thinnings should be made to reduce density and promote more rapid growth, following the same selection as before but favoring red spruce still more because of its quality of persistent growth.

Balsam and white spruce should only be grown on the better sites. On these balsam is sufficiently immune to budworm feeding up to 40 years to leave a well-stocked stand, though several years' increment will be lost and the rotation lengthened.

In the spruce swamp type, where reproduction is very good and balsam practically negligible, some form of selection system is advocated which would aim to remove mature and less thrifty trees, giving room for younger trees which grow more rapidly. Further study is needed in this type to determine the causes of periodic cycles of rapid growth in older stands and the conditions favoring rapid growth observed in younger stands following fires.

Any recommendations in hardwood mixtures are dependent on the possibilities of utilization of the hardwoods. Since hardwoods are only a protection to the softwoods while the latter are overtopped, and since once the softwoods gain dominance the mortality of balsam from the budworm is as high or higher than in pure softwoods, even greater care will be necessary to handle these mixtures successfully.

In the birch and poplar type, which is a transitional stage in the formation of the spruce flat type, the hardwoods should be regarded as purely a shelter, and the earlier the conifers are liberated the better.

In the yellow birch and northern hardwood types, where the softwoods grow very rapidly and where practical conversion to a softwood type will probably be an impossibility, efforts should be made to utilize or dispose of as much of the hardwoods as possible to liberate higher proportions of softwoods.

In hardwood mixtures balsam is most susceptible as a free tree and white spruce as an overtopped tree, which demands early cutting of free balsam and early liberation of overtopped white spruce. The spruces, particularly white spruce, should al-

ways be favored in preference to balsam.

Mature softwood types were not studied in detail, though it is believed methods involving clear cutting with the object of securing more uniform second-growth stands in compartments of varying ages are more desirable. The history of these northern forests in the past has been largely the history of burns, the resulting second growth producing relatively more budworm-resistant forests, with lower percentages of balsam. There may be a lesson in this, suggesting periodic clear cutting, a case somewhat analogous to the better results secured by periodic renewal of coppice forest by seedling trees.

Diversified forests, both as to age classes and types, will aid in lowering the momentum of budworm outbreaks and result in less general and disastrous devastation.

These recommendations are not to be considered as applicable to the Laurentian region. The entirely different silvicultural characteristics of the spruce of this region, the great difficulty of securing spruce reproduction, and the prolificness of balsam will demand different methods.

SUMMARY

The study herein reported indicates that there is a definite correlation between the mortality occurring in spruce and fir stands (from spruce budworm defoliation) and the rate of growth of these stands prior to attack. The more rapid the rate of growth as expressed in diameter increment the lower the resulting mortality under equal conditions of feeding. This relation between the effects of budworm feeding and previous vigor is a more tangible quantity than any of the other factors heretofore considered and should serve as a practical basis for preventive measures through proper silvicultural practices which maintain rapid growth.

A comparison of the rate of growth of trees surviving budworm attack shows that the diameter growth for the 10-year period following the first year of feeding is only about one-half that of the preceding 10 years.

It was found that in hardwood types the immunity of softwoods was proportional to the protection of the overstory of hardwood foliage. The percentage of mortality among dominant softwoods in mixed stands was as high as in pure softwood stands.

REVIEW OF THE NEMATODE GENERA SYNGAMUS SIEB. AND CYATHOSTOMA E. BLANCH.[1]

By EDWARD A. CHAPIN

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INTRODUCTION

Following the collection of a species of Cyathostoma from the red-tailed hawk (*Buteo borealis*) the writer has found it necessary to go into the question of the various described species of Syngamus and Cyathostoma in order to determine the status of the above-mentioned form. This investigation has shown that the species from the hawk is apparently new to science and in addition it has brought out several facts relating to the species of these two genera that seem to render desirable a general taxonomic review of the genera. The only previous paper of a similar scope is one in Russian by Skrjabin (1915), which is not readily available to most workers.

The collections of the U. S. National Museum contain abundant material for study of three species; H. A. Baylis, of the British Museum (Natural History), has kindly lent two pairs of a worm from an Old World corvine bird, and Herbert Fox, of the Zoölogical Society of Philadelphia, through F. D. Weidman, has submitted for study a series of specimens from three avian hosts. Thanks are extended for this coöperation and to B. H. Ransom for his helpful criticism.

The differential characters of value in the diagnosis of species of these genera have not as yet been well formulated and the separation of species on the basis of existing descriptions is in some cases difficult or impossible. Leiper (1913) in a paper on *Syngamus kingi* has made a useful contribution toward the formulation of characters that may be employed in the recognition of species. Though his paper has to do with the genus Syngamus only, some of his differentials may be applied to Cyathostoma equally well. He suggests as diagnostic or differential characters the following: (1) Relative position of buccal capsules in paired specimens; (2) relative length of esophagus to that of the body; (3) size and armature of mouth capsule; (4) relation of axis of buccal capsule to that of body; (5) outline of optical section of chitinous wall of capsule; (6) size of spicules; (7) configuration of posterior end of body of female; (8) site of excretory pore.

The first of these differentials of course can be used for only the species of Syngamus and then must be used with discretion. In young specimens of *S. trachea* the capsule of the male may be as much as half the length of the male in advance of the capsule of the female. On the other hand, there are before the writer gravid specimens of this worm from the turkey in which the capsule of the male is only slightly in advance of that of the female. This variation is due to the fact that the male changes little in length after maturity is reached, while the developing eggs cause apparent growth in length of the female. The second character mentioned by Leiper is apparently valid so far as the males are concerned, but is influenced in the case of the females by the factor mentioned above. The other differentials noted by Leiper appear to hold for both sexes at any age, with the possible exception of the last. The excretory pore is subject to a slight apparent migration with the egg development.

On the other hand, Leiper does not consider the bursa of the male. In the species of Cyathostoma there is no trouble involved in an examination of this organ. In Syngamus, with reasonable care, a preparation of the bursa of the male can be made without serious damage to the female worm. If with a sharp knife the genital cone of the female worm is removed from the body, the male worm with bursa entire comes away at the same time. A few minutes' careful work with a needle suffices to remove from within the bursa the fragments of the female genital cone, and the male may be then treated as any strongyle. If the pair of worms has been carried into glycerine before this operation is at-

[1] Received for publication June 24, 1924, issued June, 1925.

tempted, there is no extrusion or displacement of the female organs.

The following specific names have been applied to members of the two genera but are discarded. The reason for discarding each name is noted.

Syngamus bovis Willach 1896e. Railliet has stated (1897a) that this "species" is based on an artifact and not on a worm.
Syngamus coelebs Schlotthauber 1860a. This is a nomen nudum, based on certain worms from *Falco lagopus*.
Syngamus laryncheus Smit 1922. Lapsus calami for *S. laryngeus* Raill.
Syngamus major Smit 1922. Not distinct from *S. laryngeus* Raill.
Syngamus minor Smit 1922. *S laryngeus* Raill. renamed.
Syngamus mucronatus Schlotthauber 1860a. Nomen nudum, based on worms from *Picus canis* and *P. major*.
Strongylus pictus Creplin 1849a. *Syngamus trachealis* Sieb. renamed.
Syngamus primitivus Molin 1861a *Fasciola trachea* Mont. renamed.
Syngamus pugionatus Schlotthauber 1860a. Nomen nudum, based on worms from *Corvus pica* and *Sturnus vulgaris*.
Syngamus sclerostomum Molin 1861a. *Strongylus variegatus* Creplin renamed.
Sclerostomum syngamus Diesing 1851a. *Syngamus trachealis* Sieb. renamed.
Syngamus trachealis Sieb. 1836a. *Fasciola trachea* Mont. renamed.

There remain 11 apparently valid species which have been assigned to these genera. One at least of these, *Syngamus kingi* Leiper, appears to be out of its usual host and may be found to be synonymous with a previously described form. Another, *S. nasicola* Linst., may prove to be equal to *S. laryngeus* Raill.

SYNGAMUS VON SIEBOLD

Syngamus Sieb., 1836, Arch. Naturg., (Jahrg. 2) 1: 105–116

Generic characters. — Strongylidae; sexes permanently joined in copula; buccal capsules of both sexes large, heavily walled, furnished at the base with eight or nine teeth arranged about the center, the teeth of two distinct sizes. Excretory pore anterior to the esophago-intestinal junction; esophagus moderate, pestle-shaped. Males with thick-walled bursa; bursal rays short and thick; spicules small to very small (150 μ to 25 μ). Vulva of female in the anterior third of the body length; tip of female tail blunt or acute. Eggs moderate in size, operculated after deposition.

Habitat.—In the respiratory tract of birds and mammals.

Type species.—(*Syngamus trachealis* v. Sieb.) = *Fasciola trachea* Mont.

Six valid species which may be referred to this genus are recognized in this paper. The appended key emphasizes the points of difference. Two other species are considered doubtful and are treated at the end of the list.

KEY TO SYNGAMUS

1. Species infesting mammals 2
   Species infesting birds 3
2. Ovarian-uterine complex reaching just beyond the middle of the length; spicules 25 μ long, host *Bos taurus* ..... *S. laryngeus.*
   Ovarian-uterine complex reaching nearly to anus ..... *S. dispar.*
3. Branches of dorsal ray simple, not subdivided 4
   Branches of dorsal ray divided in apical portion 5
4. Spicules subequal, 50 μ long, host *Nucifraga caryocatactes* ..... *S. parvus.*
   Spicules distinctly unequal, right spicule 79 μ, left spicule 70 μ long, host *Corvus brachyrhynchos* ..... *S. gracilis.*
5. Each branch of dorsal ray bifurcate, spicules 150 μ long, host *Phalacrocorax carbo* ..... *S. microspiculum.*
   Each branch of dorsal ray trifurcate, spicules 60 μ long, hosts gallinaceous birds ..... *S. trachea.*

SYNGAMUS LARYNGEUS Railliet (pl. 1, fig. 4; pl. 2, figs. 12, 13, 16; pl. 4, fig. 44).

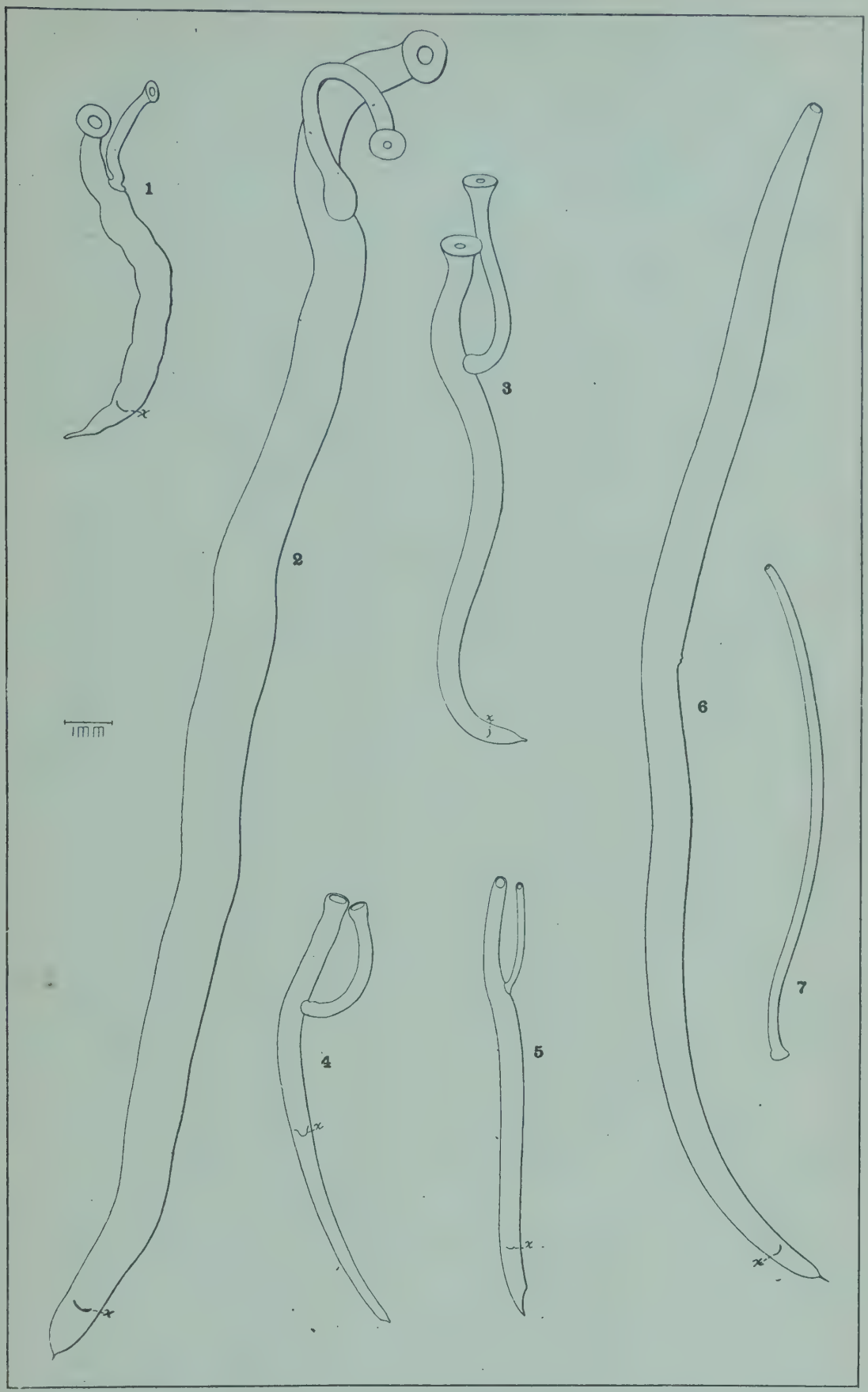
Syngamus laryngeus Raill, 1899, Compt. Rend. Soc. Biol. [Paris] (XI) 1: 18–21; Sheather and Shilston, 1920, Pusa [India] Agr. Research Bul. 92, 8 p.
S. laryncheus Smit, 1922, Deut. Tierärztl. Wchnschr. 30: 506–507. (Lapsus for *laryngeus.*)
S. Laryncheus minor Smit, 1922, Deut. Tierärztl. Wchnschr. 30: 507. (*laryngeus* Raill. renamed.)
S. major Smit 922, Deut. Tierärztl. Wchnschr. 30: 507.

The material representing this species which is available for study consists of four lots, all from *Bos taurus*, as follows: One pair from Cochin China, received from A. Railliet in 1899; many pairs from Manila, Philippine Islands, received from William Boynton in 1914, and two lots from Rio Piedras, Porto Rico, received from J. Bagué in 1917. The specimens, a pair in copula, from Railliet may be considered in the light of type material.

Smit described spicules reaching from the end of the esophagus backward, presumably to the bursa. It is uncertain just what organ has been mistaken for spicules; there is, however, no doubt that the organs described are not spicules.

Six pairs have been examined in detail, one from Cochin China, three from Manila, and two from Porto Rico. There appears to be considerable variation in the actual sizes of the various organs, which, however, are not coordinated with the geographical distribution.

MALE.—The total length is from 2.6 to 3.6 mm. Sheather and Shilston report males as long as 5 mm. The buccal capsule in the Cochin China specimen is 0.23 by 0.27 mm.; in the others it is larger, attaining the size of



1. *Syngamus parvus* n. sp. Outline of pair (female gravid).
2. *Syngamus trachea* (Mont.). Outline of pair (female gravid).
3. Idem. Outline of pair (female immature).
4. *Syngamus laryngeus* Raill Outline of pair (female gravid)
5. *Syngamus dispar* (Molin). Outline of pair. (After Diesing.)
6. *Cyathostoma americana* n. sp. Outline of female (gravid).
7. Idem. Outline of male.

(x=posterior extent of uterine coils)

0.30 by 0.36 mm. in one from Manila. The esophagus is from 0.66 to 0.89 mm. long and from 0.7 to 0.25 mm. wide at the point of greatest width (cardiac dilation). The bursa is nearly circular. On the ventral line there is a notch which is more or less completely closed by a small lobe. The supporting rays may be distinguished only under extremely favorable condition and the form of their terminations has not been made out. From examination of several specimens it is evident that there is a complete set of rays; the dorsal, apparently undivided with the externo-dorsal rays are grouped closely together. The postero-lateral, medio-lateral, and externo-lateral rays form a compact group at the extremity of the transverse diameter. The ventro-ventral ray is close to the insertion of the ventral lobe, while the latero-ventral ray is just beside it.

In two specimens, which after dissection were in suitable condition for study, spicules were found. The small size of the spicules explains the failure of other observers to find them. They are similar in shape, about 25 μ long, and lie just inside the cloacal opening. The opening itself appears to be supported by a chitinous ring, perhaps a modified gubernaculum. Except for the ring, no structure was seen that could be identified as a gubernaculum.

FEMALE.—The position of the vulva in respect to the total length of the worm is less variable toward maturity in this species than in *S. trachealis*. This is due to the fact that the uteri do not extend much farther behind the vulva than they do in front of it. Thus in gravid specimens the distortion due to the ripe eggs is nearly evenly balanced before and behind, and the ratio obtaining in mature but not gravid worms is maintained in the gravid specimens. In the specimens measured, the ratio of the distance between the anterior end and the vulva to the total length of the worm varies from 1: 4.64 to 1: 4.69. Owing to the anterior distribution of the uteri, the mature worms assume a distinctive tapering form, the posterior third being perceptibly thinner than the anterior two-thirds.

A very young pair (female 3.58 mm. in length) shows certain features of interest. The spicules are easily seen without dissection. The vagina is very short; the bifurcation of the uteri occurs within the area covered by the male bursa. The uteri make a short anterior loop and then proceed posteriorly to a point less than half the distance from the vulva to the tail. The ovaries occupy most of the space between this point and the posterior extremity. The vulva divides the total body length of the female as 3 : 5 or 1 : 1. 66

It appears that in this species the ovarian tissue degenerates after the eggs are discharged into the uteri, for in gravid specimens the ovaries are not perceptible.

Host.—Bos taurus, doubtfully from Homo.

Location.—Larynx.

Geographic distribution.—Indo-Malayan region, Porto Rico, Brazil?.

Travassos (1922) reports a case of *S. laryngeus* in man (Brazil). There is no evidence given to show whether he was dealing with this species or another.

SYNGAMUS DISPAR (Diesing) (pl. 1, fig. 5).

Sclerostoma dispar Dies. 1851, Syst. Helm., 2: 303; Dies., 1857, Denkschr. Akad. Wiss., Wien, Math.-naturw. cl., 13 (abt. 1): 16.
Sygnamus dispar Molin., 1861, Mem. R. Inst. Veneto Sci., Litt. ed Arti 9:565.

There is unfortunately no material of this species available nor is there a good or even a moderately good description extant. The available figures (Diesing) cover one or two important points not mentioned in the description. The following is a summary of the specific characters, taken from all available sources:

Mouth opening terminal; buccal capsule with ribs, presumably ending at the base in lancetlike teeth; bursa of male rather more strongly developed than in *S. laryngeus;* spicules probably minute (not described); vulva of female at about anterior fourth; coils of uterine complex reaching to posterior seventh of the total length; tail short, stoutly conical; anus just before tail. Length of male, 5–7 mm.; of female, 20–27 mm.

Host.—Felis concolor.

Location.—Trachea.

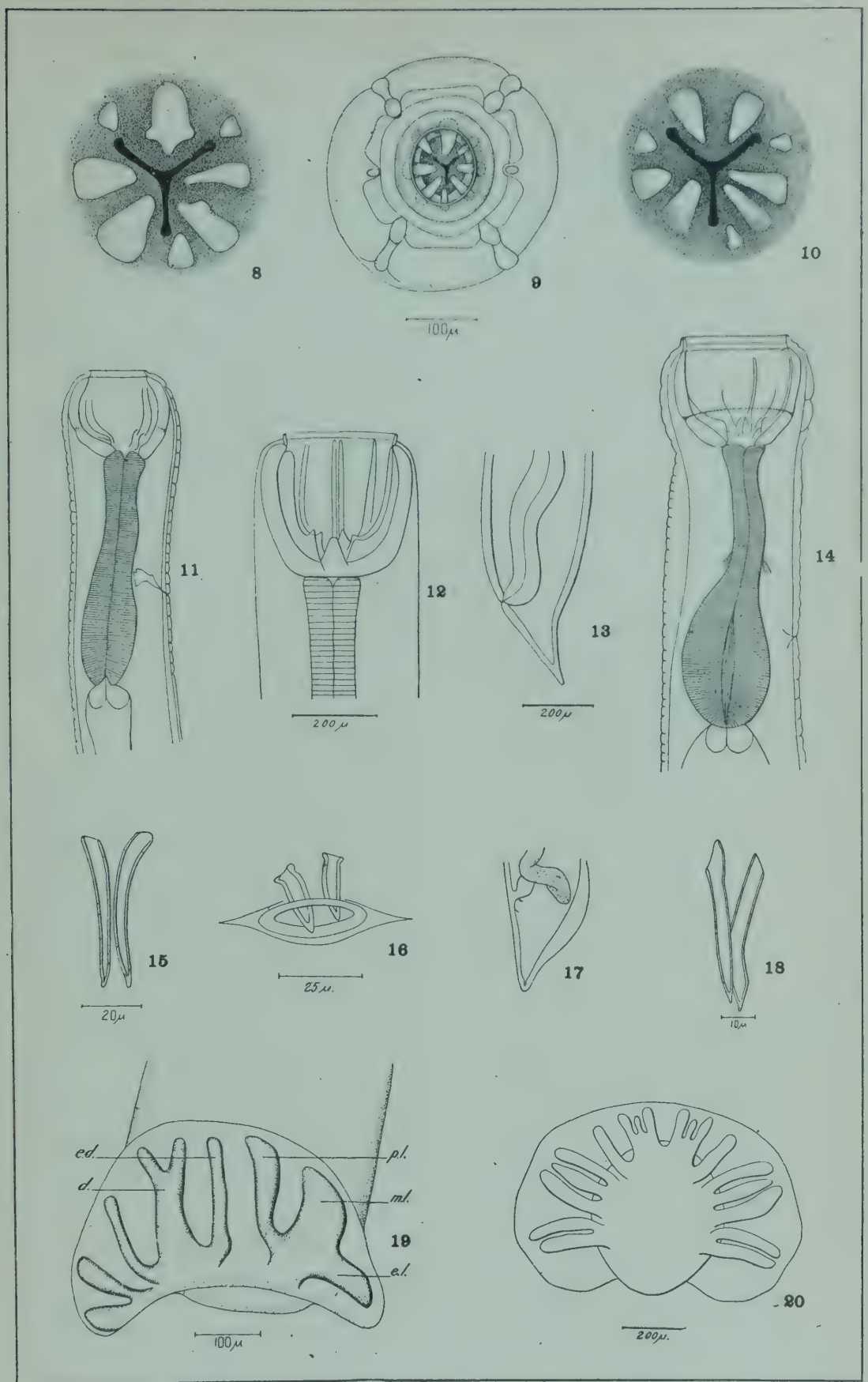
Geographic distribution.—Brazil.

Though little concerning this species is known, still it is highly probable that the species is valid. The only possible conflict is with *S. kingi* Leiper. A discussion of the relationship is included under that species.

Syngamus parvus n. sp. (pl. 1, fig. 1; pl. 2, fig. 15, 19).

Similar in general shape to *S. trachea* but much smaller and with other male secondary sexual characters.

MALE.—About 2.4 mm. long, 0.22 mm. in diameter, cylindrical, slightly



8. *Syngamus trachea* (Mont.). Arrangement of teeth in eight-toothed (normal) form.
9. Idem. Front view of head.
10. Ibidem. Arrangement of teeth in nine-toothed form.
11. *Syngamus kingi* Leiper. Side view of anterior end of male. (After Leiper.)
12. *Syngamus laryngeus* Raill. Side view of anterior end of male.
13. Idem. Side view of tail of female.
14. *Syngamus kingi* Leiper. Side view of anterior end of female. (After Leiper.)
15. *Syngamus parvus* n. sp. Spicules.
16. *Syngamus laryngeus* Raill. Spicules.
17. *Syngamus kingi* Leiper. Side view of tail of female. (After Leiper.)
18. *Syngamus trachea* (Mont.). Spicules.
19. *Syngamus parvus* n. sp. Dorsal portion of bursa of male.
20. *Syngamus trachea* (Mont.). Arrangement of rays in bursa of male

constricted in neck region. Buccal capsule heavily chitinized, 178 μ in depth, 207 μ in its greatest inside diameter, walls 29 μ thick. Buccal teeth and circumoral papillae as in *S. trachea*. Esophagus about 326 μ in length. Nerve ring? Excretory pore? Bursa 350 μ in diameter; ventral rays short, stout, and approximate; lateral rays stout; medio-lateral ray much so; externo-lateral ray arising from the side of the medio-lateral; postero-lateral ray slender in comparison with the other two. Externo-dorsal ray more slender and parallel than postero-lateral; dorsal trunk bifurcated near tip, each bifurcation simple. Spicules short, about 49 μ, similar in shape to those of *S. trachea*.

FEMALE.—About 7.8 mm. long, 0.65 mm. in diameter at widest part. Neck region just back of buccal capsule about 0.35 mm. in diameter. Buccal capsule 300 μ in depth, 440 μ in average diameter. Buccal teeth as in *S. trachea*. Nerve ring? Excretory pore? Esophagus short and thick. Vulva in gravid worm dividing total length into ratio as 1:5.2; uterine coils extending backward to 1.25 mm. from apex; anus subterminal; tip of tail very blunt. Eggs 74 by 44 μ, not yet segmented in uterus.

Host.—Nucifraga caryocatactes.

Habitat.—Trachea.

Geographical distribution.—Not stated for present specimens. (Host occurs in Europe.)

Syntypes.—British Museum (Natural History), part of lot No. 92.11.4.1–3. One pair deposited in U. S. N. M. Helm. Coll., No. 26098.

Through the kindness of H. A. Baylis, the author has had the opportunity to study the worms upon which the foregoing description is based. Unfortunately, the preservation of the specimens is not of the best, but it is believed that sufficient differences can be made out to warrant the setting apart of the material from Nucifraga as a species distinct from *S. trachea* (Mont.). Thanks are extended to Baylis for securing the gift of one pair for the National Museum collection.

Both of the species of Syngamus described in this paper from corvine hosts agree in having the dorsal ray of the male bursa branched, the branches simple. In the present species the branching occurs beyond the middle of the length. In the American form, *S. gracilis*, the bifurcation is basal, resulting in a total suppression of the common dorsal trunk.

Syngamus gracilis n. sp. (pl. 4, figs. 38, 40, 42).

Syngamus trachealis Wied., (pars), Fox, 1923, Disease in captive wild mammals and birds p. 650.

Superficially resembling *S. trachea* (Mont.), but smaller and more slender. Male from 3 to 3.3 mm. in length, cylindrical, thickness 270 μ. Buccal capsule heavy, its wall 45 μ thick; the diameter of the lumen of the capsule near the upper edge is about 200 μ, the depth 165 μ. Buccal teeth as in *S. trachea;* the largest have an altitude of 45 μ. The esophagus is nearly 600 μ long, clavate, its greatest diameter 135 μ. Nerve ring, excretory pore, and cervical papillae not seen.

Caudal bursa narrow and deep; branches of the dorsal ray 150 μ long; externo-dorsal ray slightly shorter, measuring 132 μ. The lateral rays are mutually contiguous, parallel, and about the size of either branch of the dorsal. The ventral rays are smaller and more slender. The spicules are distinctly unequal; the right is bent and measures 79 μ long, while the left is nearly straight and is but 69 μ in length.

The female is from 8 to 11 mm. long and is proportionately stouter than the male. The buccal capsule is relatively enormous, its internal dimensions being 525 μ wide by 300 μ deep. The wall is 50 μ thick. Teeth as in *S. trachea*. The esophagus is 825 μ long and 225 μ thick at the point of its greatest diameter, just before the esophago-intestinal valve. Other structures in the neck region not seen. The vulva is 1.4 mm. behind the anterior end or at about the anterior eighth of the body length. The vagina is longer than in related species, and both branches of the uterus proceed backward. The anus is about 300 μ in front of the very blunt posterior extremity. The eggs range when mature from 79 to 83 μ in length by 40 to 46 μ in width.

Host.—Corvus brachyrhynchos.

Location.—Trachea.

Geographic distribution.—Philadelphia Zoölogical Park, Nov. 14, 1914.

Type and allotype.—U. S. N. M Helm. Coll. No. 26127. Paratypes in collection of the Zoological Society of Philadelphia.

This species is more closely related to *S. parvus* from *Nucifraga caryocatactes* than to *S. trachea* of the gallinaceous birds. Yet it is in all probability the form which normally occurs in the

American crow. It is further probable that *S. trachea*, which can hardly sustain itself in a host as close phylogenetically to the turkey as the domestic fowl is, cannot under even the most favorable circumstances reach maturity and egg production in the crow. From this it seems probable that the crow is not, as often stated, a reservoir for the turkey and chicken gapeworm.

SYNGAMUS MICROSPICULUM Skrjabin (pl. 4, fig. 39).

Syngamus microspiculum Skrjabin, 1915, Vestnik Obshch. Vet. 27: 646.

Through the kindness of Robert J. Formad, the writer is able to offer the following translation of Skrjabin's original description:

MALE.—Cylindrical in shape, dark brownish in color, 3.9 to 4 mm. in length. In the region of the posterior portion of the esophagus the body is 0.29 mm. in diameter, while just before the bursa the diameter is 0.27 mm. The mouth capsule is 0.17 mm. deep by 0.22 mm. broad and is provided within at the base with three pointed triangular teeth, each 0.07 mm. long. The esophagus is 0.58 mm. long and is flask-shaped at its posterior end. The bursa of the male is very characteristic; the dorsal ray is dichotomously divided, the externo and postero-lateral rays form a compact group, and the latero-ventral and ventro-ventral rays are approximate. In this species the spicules are unusually characteristic. They are two in number, of similar size, and their length measures 0.15 mm.[2] As will be seen later, no one species of Syngamus has such small spicules, and for this reason this organ serves as the principal diagnostic sign of the species. The spicules have a thickened base and taper toward the posterior end; their color is brownish.

FEMALE.—Length 11 mm.; diameter in the region of the posterior portion of the intestine 420 μ, in the region of the vulva 500 μ, and near the anus 250 μ. The buccal capsule is broader than deep, its diameter being 250 μ and its depth 340 μ. The clavate or bottle-shaped esophagus is 765 μ long. The vulva is situated 4.45 mm. from the anterior end. The eggs are 75 μ long by 48 μ in transverse diameter. Their shape is very characteristic. One side of the egg is flat, the other hemispherical. The tail of the worm is acutely conical, similar to that of *S. variegatus* Crepl.

Syngamus microspiculum is distinguished from all described species by the following: (1) Size of spicules; (2) there are three teeth in the buccal capsule; (3) the position of the vulva; (4) the shape of the eggs.

Host.—Phalacrocorax carbo.

Location.—Trachea.

Geographic distribution.—Turkestan.

Skrjabin's description of three buccal teeth would seem to demand confirmation. Furthermore, the statement that the spicules of this form are the shortest known in the genus is not accurate. At the time Skrjabin's paper was written the spicules of *S. trachea* were already described as 60 μ in length. The dorsal ray of the bursa and the spicules are, however, ample to differentiate this species from any other known.

SYNGAMUS TRACHEA (Montagu) (pl. 1, fig. 2; pl. 2, figs. 8 to 10, 18, 20).

Wissent., 1799, Med. & Phys. Jour. [London 2: 204–205.

Fasciola trachea Mont., 1811, Mem. Werner. Nat. Hist. Soc. Edinb., (1808–10) 1: 176–193.

Syngamus trachealis, Sieb., 1836, Arch. Naturg. (Jahrg. 2), 1 (1): 105–116; Ransom, 1921, U. S. Dept. Agr. Bul. 939, 13 p.; Ortlepp, Jour. Helminthology, 1: 119–140.

Syngamus trachea (Mont.) Chapin, 1924, present paper.

In accordance with the International Code of Zoological Nomenclature, the name of this species should be *trachea* rather than *trachealis*, the former having some years' priority.

Much stress has been laid in the past on the position of the vulva as a taxonomic character. When used with caution and with due consideration for the age of the specimen, it is of considerable value. However, it must be recognized that as the worm ages and the uteri become packed with eggs there is a stretching, not only in the diameter of the worm but in the length. For instance, in the present species the vulva is well forward and the uterine complex is largely behind it. In young worms, quite immature so far as egg production is concerned, the ratio of the distance from the anterior end to the vulva to the total length of the worm is 1 to 3.25. In specimens showing a few eggs the ratio changes to 1 : 4.25, while in gravid specimens the ratio may become as

[2] In a later paper (Ann. Mus. Zool. Acad. Imp. Sci., Petrograd (1915) 1916, 20: 467–471), Skrjabin has redescribed this species and has given notes on certain other species of the genera considered here. The noteworthy difference between the two descriptions is that the spicules are described first as 150 μ long, later as 115 μ. Either statement may be true but it is impossible to state which was the intended figure by a study of the context. Also in the later paper the length of the spicules of *Syngamus trachealis* (= *S. trachea*) is given as 0.69 mm., a figure about ten times greater than that usually accepted. This is undoubtedly a typographical error for 69 μ.

high as 1 : 5. That the variation here noted is due to the presence of large numbers of eggs is shown by a corresponding study of *S. laryngeus* Raill., where the uterine complex extends hardly farther behind the vulva than it does in front of it.

Leiper has placed considerable emphasis on the relation of the axis of the buccal capsule to that of the body of the worm, stating that the capsule is turned dorsally. In old pairs it is true that the mouth opening is directed dorsally, but in young worms the mouth is directed anteriorly. The capsule is at all times terminal; any changes in the relative directions of the axes is due to a flexure of the neck region.

The teeth at the base of the buccal capsule have heretofore been described as eight in number but with no statement as to the relative sizes of the teeth. The normal form shows eight teeth, as follows: A large medium dorsal tooth which may or may not show two cusps. On either side of this tooth there is a very small submedian dorsal tooth. There are two large (always unicuspid) lateral teeth, one on each side, and two large submedian ventral teeth, each adjacent and similar to a lateral tooth. The ventral tooth is the smallest of all.

Occasionally there are nine instead of eight teeth, in which case the median dorsal is divided to form two teeth.

The spicules of ten males selected at random from material taken from both turkey and fowl vary in length from 57 μ to 64 μ. It is probable that reports of 140 μ spicules in this species refer to another species.

Host.—Meleagris gallopavo, Gallus domesticus.

Location.—Trachea.

Geographic distribution.—North and South America, Europe, Africa, Australia.

As Ransom has shown, the turkey should be considered the true host of this parasite. Chickens are readily susceptible to infection only when very young, and infection usually proves fatal. If the chicken survives the infection it loses its parasites within a short time, so that there is little opportunity for spread of infection from chickens. Available evidence indicates that in the absence of turkeys gapeworm infection is rare among chickens. Under these circumstances it would seem desirable to compare more carefully than appears to have been done specimens of gapeworms that have been collected from various species of birds other than the Galliformes to determine whether they are really *Syngamus trachea.*

SYNGAMUS KINGI Leiper (pl. 2, fig. 11, 14, 17).

Syngamus kingi Leiper, 1913, Trans. Soc. Trop. Med. & Hyg. [London] (1912–13), 6: 265–297.

The material on which this species is based was collected from *Homo sapiens* in St. Lucia, West Indies. From Leiper's description one may say definitely that the species is not *S. laryngeus* Raill., since the coils of the uterine complex reach to just in front of the anus; nor can it be *S. trachea*, for in that case the opening of the mouth capsule is turned dorsally instead of being terminal. The possibility that *S. dispar* and *S. kingi* are synonymous is not excluded. As has been pointed out, the transverse level of the month capsules in all species I have examined varies according to the age of the specimens, and the differentiation between *S. kingi* and *S. dispar* made by Leiper on the basis of this character accordingly seems open to question.

SYNGAMUS NASICOLA Linst.

Syngamus nasicola Linst. 1899, Mitt. Zool. Samml. Mus. Naturk. 1 (2): 18.

MALE.—5.6 mm. in length, 0.47 mm. in thickness. Buccal capsule 320 μ deep by 480 μ in breadth, with six ribs proceeding from the teeth on the internal wall. Esophagus ten forty-sixths of the body length.

FEMALE.—20.6 mm. long, 0.87 mm. thick. Esophagus one fifty-first (sic.) of the body length; tail one fifty-second of the body length and acutely conical. Vulva divides body as 3: 10. Eggs 88 by 46 μ.

Host.—Cervus rufus; Capra hircus.

Habitat.—Posterior nares, nasal cavity.

Geographical distribution.—Brazil (Rio Grande do Sul), Africa (Cameroon).

In the foregoing description, which contains all of the pertinent points mentioned in the original, there is only one point that serves to differentiate this species from *Syngamus laryngeus.* That point is the proportional dimensions of the buccal capsule of the male. If von Linstow was correct in saying that the capsule measured was that of the male, the capsule of the female should be very shallow and the two species are probably distinct. On the other hand, the figures cited are approximately those of the female capsule of *S. laryngeus.* The other

characters mentioned apply to *S. laryngeus*, and for the present it seems best to consider von Linstow's species as doubtful. As the descriptions of *S. laryngeus* Raill. and *S. nasicola* Linst. appeared almost at the same time, and as von Linstow made no mention of Railliet's species, it is probable that he was unaware of the existence of *S. laryngeus* Raill. Certainly the case appears to be that of the simultaneous publication of the same species under two specific names.

CYATHOSTOMA E. BLANCH.

Cyathostoma E. Blanch. 1849, Ann. Sci. Nat., Zool. (III) 11: 182.

Generic characters.—Strongylidae, sexes not joined in permanent copula,; buccal capsules of both sexes large heavily walled, furnished at the base with six or seven teeth arranged about the center, the teeth of two distinct sizes. Excretory pore as in Syngamus. Esophagus as in Syngamus. Males with normal strongyliform bursa, rays slender and sometimes (dorsal ray) branched, spicules long (more than 400 μ) and filiform. Vulva of female variable in position, sometimes in anterior third, sometimes median or slightly postmedian; tip of female tail acute. Eggs moderate in size, operculated after deposition.

Habitat.—In respiratory tract of birds.

Type species.—*Cyathostoma lari* E. Blanch.

Seven species, apparently all valid, may be referred to the genus *Cyathostoma*. At present, however, it is necessary to rely on certain unsatisfactory characters in order to form the key to species which appears below.

1.	Vulva at or near the anterior third of the body	2
	Vulva near the middle of the body	3
2.	Eggs 90 μ by 60 μ; spicules 600 μ long; hosts Anseriform birds	*bronchialis.*
	Eggs 80 μ by 55 μ; spicules 650 μ long; host *Casuarius galeatus*	*boularti.*
	Eggs 80 μ by 40 μ; spicules? long; hosts Ciconiform birds	*variegatum.*
	Eggs 56 μ long; spicules 500 μ long; host *Tadorna tadorna*	*tadornae.*
3.	Male unknown; female small, up to 13 mm. long; in orbital cavity of *Larus ridibundus*	*lari.*
	Male known, female larger	4
4.	Spicules of male 490 μ long, gubernaculum 67 μ long; female up to 30 mm. long; in thoracic air sac of *Buteo borealis*	*americanum*
	Spicules of male 660 μ to 720 μ long, gubernaculum 92 μ; female up to about 20 mm. long; in trachea of *Coscoroba coscoroba*	*coscorobae.*

CYATHOSTOMA BRONCHIALIS (Muehlig) (pl. 3, fig. 32 to 34.)

Syngamus bronchialis Muehlig, 1884, Deut, Ztschr. Thiermed., 10: 265; Raill., 1898, Compt. Rend. Soc. Biol. [Paris] (X) 5: 400.
Cyathostoma bronchialis (Muehlig) Chapin, 1925, present paper.

MALE from 4 to 5.8 mm. in length, 260 μ in diameter, slightly attenuate anteriorly. Esophagus about 350 μ long. Caudal bursa entire; main trunk of the dorsal ray longer than the branches, which diverge sharply and are irregular in outline. Externo-dorsal and externo-lateral rays short, simple, subequal, each slightly reflexed at tip. Lateral rays arising from a common trunk, medio and postero-lateral rays separate, their tips approximate. Ventral rays separate and equal, the rays about as long as the common trunk; spicules thin, 510 to 620 μ long; internal margin fimbriate; basal portion feebly dilated; apices incurved and pointed.

FEMALE from 16 to 31 mm. long, 700 to 900 μ in diameter. Buccal capsule 325 μ broad by 205 μ deep. Esophagus very variable in length, according to age of worm, from one-thirteenth to one-thirtieth of the total length. Tail short, conical, sometimes curved. Anus 160–300 μ before the extreme apex. Vulva slightly prominent, just in front of the anterior third. Eggs narrowly ovoid, 74 to 83 μ long by 49 to 62 μ wide, with an inconspicuous operculum at the narrow pole.

Hosts.—Domestic geese, swan.

Location.—Trachea and bronchi.

Geographic distribution.—Europe and North America.

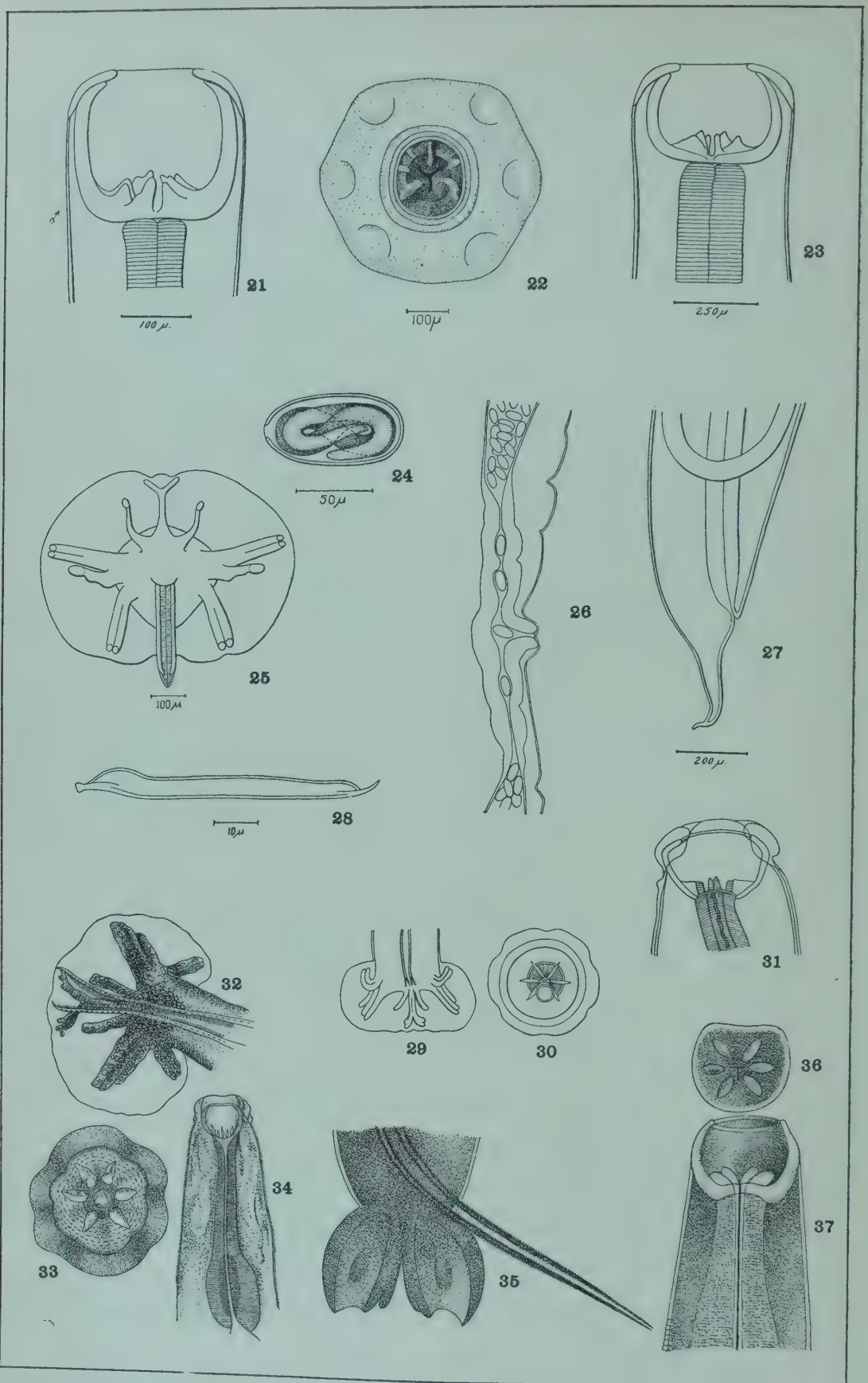
There is a single female in the collection from a swan, sent from Ithaca, N. Y., by W. A. Hagan, April, 1920. Although not in the best condition for study, it does not appear to be different from the above.

CYATHOSTOMA BOULARTI (Mégnin) (pl. 3, fig. 35 to 37).

Sclerostoma boularti Mégnin, 1884, Jour. Anat. et Physiol., 20=457.
Syngamus boularti (Mégnin) Raill., Compt. Rend. Soc. Biol. [Paris] (X) 5 : 402.
Cyathostoma boularti (Mégnin) Chapin, 1925, present paper.

Body soft, colored bright red by intervisceral fluid; intestine spiral, much longer than the body; head narrower than the neck.

MALE 7 mm. long, 450 μ thick. Head narrow, 200 μ in diameter; buccal opening terminal, 120 μ in diameter. Body narrowly cylindrical, truncate



21. *Cyathostoma americanum* n. sp. Side view of anterior end of male.
22. Idem. Front view of head.
23. Ibidem. Side view of anterior end of female.
24. Ibidem. Egg from culture (88 days old).
25. Ibidem. Bursa of male.
26. Ibidem. Female genitalia in the region of vulva.
27. Ibidem. Side view of tail of female.
28. Ibidem. Gubernaculum.
29. *Cyathostoma variegatum* (Crep.) Bursa of male. (After Linstow.)
30. Idem. Front view of head. (After Linstow.
31. Ibidem. Side view of head. (After Linstow.)
32. *Cyathostoma bronchialis* (Muehlig). Bursa o male. (After Muehlig.)
33. Idem. Front view of head. (After Muehlig.)
34. Ibidem. Side view of head. (After Muehlig.)
35. *Cyathostoma boulart* (Mégnin). Ventral portion of bursa of male. (After Mégnin.)
36. Idem. Front view of head. (After Mégnin.)
37. Ibidem. Side view of head. (After Mégnin.)

posteriorly, terminated by a 2-lobed membranous bursa, each lobe of which is supported by five rays; spicules two, thin, each 650 μ long.

FEMALE from 18 to 20 mm. long, diameter 850 μ. Head 500 μ in diameter, with buccal opening 350 μ across. Body cylindrical, fish-hook shaped, with a short conical tail. Anal opening just before the posterior extremity; vulva prominent, situated at the anterior third of the body length. Oviparous. Eggs smooth, ovoid, 80 by 55 μ, with an opercle at the smaller end.

Host.—Casuarius galeatus.

Location.—Trachea.

Geographic distribution.—Australia. (Original lot from host in the Zoölogical Garden, Paris.)

The above is a free translation of the original description by Mégnin. No specimens are available for examination. The original figure of the bursa does not at first glance seem plausible. In mounting a specimen of *C. americanum* for examination the present writer happened to secure an almost identical picture. I believe it may be safely assumed that Mégnin failed to figure the dorsal half of the bursa which was superimposed on the worm. The portion figured shows the ventral rays centrally located, the medio and postero-lateral rays together as the lateral fields in Mégnin's figure. The apices of the externo-lateral rays appear in the figure as the isolated oval spaces, one in each lobe. From the length of the spicules, and from the host, it is, I believe, wise to consider the species as valid.

CYATHOSTOMA VARIEGATUM (Creplin) Emend. (pl. 3, fig. 29 to 31).

Strongylus trachealis Nathusius, 1837, Arch. Naturg. (Jahrg. 3) 1: 60. (nec. *Syngamus trachealis* Sieb. 1836.)
Strongylus variegatus Creplin, 1849, Arch. Naturg. (Jahrg. 15) 1: 64. (*Strongylus trachealis* Nath. renamed.)
Syngamus variegatus (Creplin) Raill. 1898, Compt. Rend. Soc. Biol. [Paris] (X) 10: 401.
Cyathostoma variegatum (Creplin) Chapin, 1925, present paper.

MALE 7.8 mm. to 9 mm.; female 13.5 mm. to 45 mm. in length. Body slightly attenuated in either direction; posterior extremity of female ending in an acute tail, that of male truncate. Mouth opening circular, very broad, leading into a buccal capsule with heavy chitinous walls. Buccal teeth six in number (?), corresponding in position with the circumoral papillae. Esophagus flask-shaped, with a long neck; in the male the esophagus is more slender. In the female the anus opens just before the tip of the tail; in the male it is within the bursa. Vulva prominent, transverse, crescentic, situated at the interior third of the body length. Eggs 80 μ by 40 μ. Bursa of male strongyliform, dorsal ray bifurcate. Spicules long, mutually similar, filiform.

Host.—Ciconia nigra.

Location.—In trachea.

The above-mentioned data are taken from the original description of Nathusius and are sufficient merely to show that the species is one of the four known forms in which the vulva lies at the anterior third. A knowledge of the exact length of the spicules would probably serve to determine the validity of one or more of the other species.

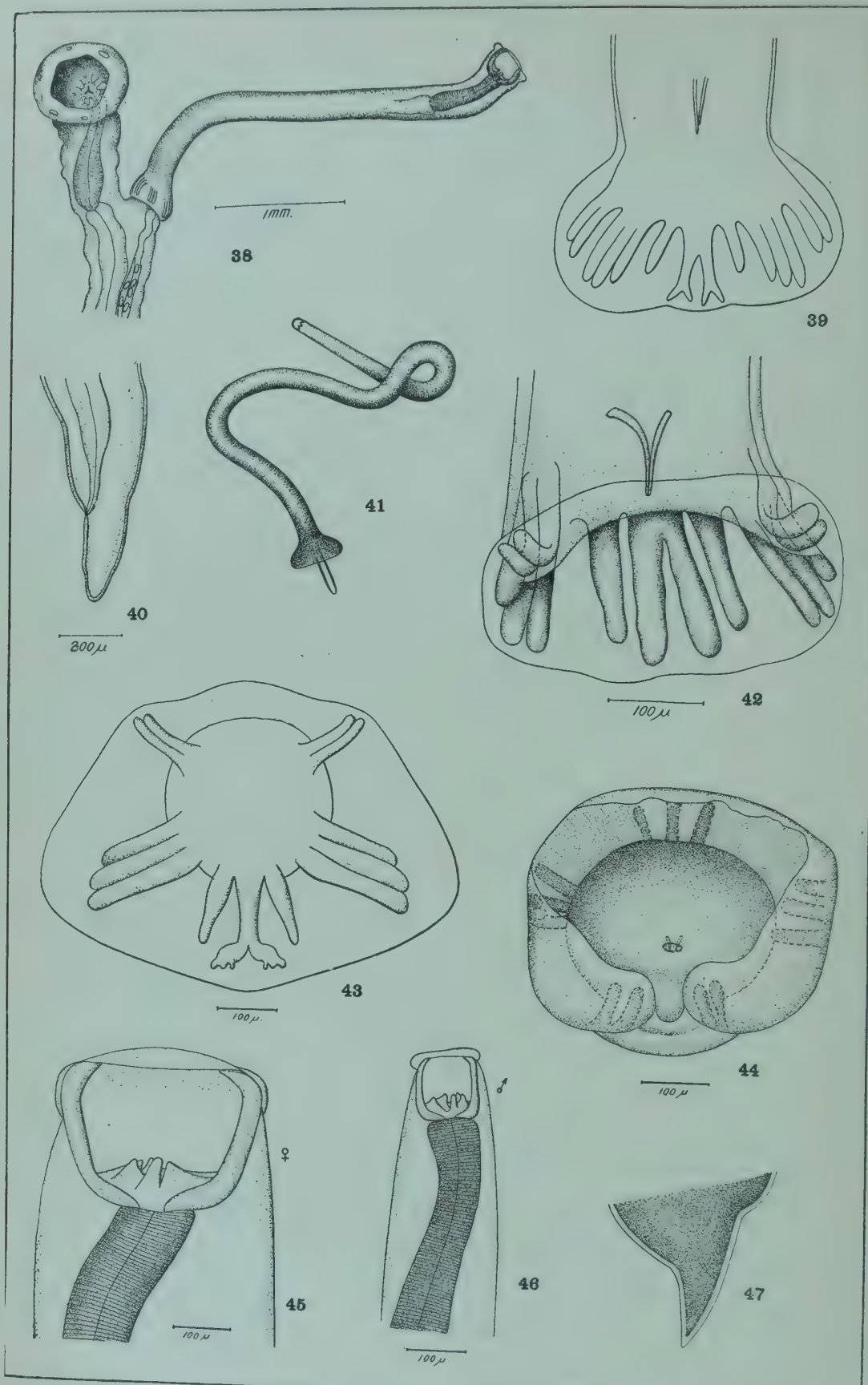
At first glance it might seem that a combination of *Cyathostoma* with *trachealis* Nathusius would be the correct name for this species, since there is no conflict with *trachealis* Sieb. Nathusius did not intend to name any species. He misidentified the material before him, and on the strength of his identification he transferred Siebold's species from Syngamus to Strongylus. While the International Code of Zoölogical Nomenclature is not explicit on the point, it would appear that for the sake of stability of nomenclature an unintentional creation of a name should be disregarded. A misidentification is not the proposal of a new name nor can it be regarded as tantamount to such.

CYATHOSTOMA TADORNAE Chatin (pl. 4; figs. 41, 47).

Cyathostoma tadornae Chatin, 1874, Ann. Sci. Nat. Paris (VI) 1 (Art. 6): 1–6.
Sclerostoma tadornæ Linst., 1878, Compend. Helm, p. 152.
Syngamus tadornae Raill., 1898, Compt. Rend. Soc. Biol. [Paris] (X) 5 : 402.

MALE.—Length 9.6 mm., form cylindrical, of uniform thickness throughout; color brick-red. Cuticle transversely striate at intervals of 20 μ, longitudinal striae apparently absent. Buccal capsule corneous and cup-shaped, furnished at its base with two pharyngeal teeth. Esophagus thick, clavate, about one-fifteenth of the body length. Body is terminated by a bursa, well developed and regular, which is supported by ten rays, four of which are simple, the remaining six being bifurcate at their apices. From within the bursa there extend two brown spicules, 500 μ long.

FEMALE.—Length 23 mm. Body of a brighter red than that of male. Head 0.9 mm. in diameter, similar to that of male. Buccal capsule as in male. Esophagus 1.8 mm. long, clavate. Vulva situated near the anterior third of the body. Uteri well developed, packed with eggs measuring in their longest diameter 0.56 mm. (sic!). Body terminated by a conical



38. *Syngamus gracilis* n. sp. Male and anterior portion of female.
39. *Syngamus microspiculum* Skrj. Bursa of male. (After Skrjabin.)
40. *Syngamus gracilis* n. sp. Posterior extremity of female.
41. *Cyathostoma tadornae* Chatin. Male, entire worm. (After Chatin.)
42. *Syngamus gracilis* n. sp. Bursa of male.
43. *Cyathostoma coscorobae* n. sp. Bursa of male.
44. *Syngamus laryngeus* Raill. Bursa of male.
45. *Cyathostoma coscorobae* n. sp. Buccal capsule of female.
46. Idem. Buccal capsule of male.
47. *Cyathostoma tadornae* Chatin. Posterior extremity of female. (After Chatin.)

process, set at an angle with the longitudinal axis of the body.

Habitat.—In the trachea of *Tadorna tadorna* L.

There is no evidence to show that the buccal capsule of this species was examined otherwise than from the side, and for that reason the statement concerning the number of buccal teeth may be questioned. There are probably six or seven teeth present, as in the other species of the genus. The size given for the egg is ten times too large, according to the figure. The magnification as given on the plate gives a length of about 60 μ.

CYATHOSTOMA LARI E. Blanchard.

Strongylus sp., Siebold, 1837, Arch. Naturg (Jahrg. 3) 1: 68.
Cyathostoma lari E. Blanch., 1849, Ann. Sci. Nat. Zool. (III) 11: 183.
Sclerostoma cyathostomum Dies., 1851, Syst. Helm., 2: 306. (*Cyathostoma lari* E. Blanch. renamed.)
Sclerostoma lari (Blanch.), Molin, 1861, Mem. R. Inst. Veneto Sci. Lett. ed. Arti, Venezia, (1860) 9: 561.
Syngamus lari (Blanch.), Ransom, 1904. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 60: 43, figs. 38–40; Linst., 1909, Süsswasserf. Deutschl., Heft 15, Parasit. Nematoden, p. 62.

Body red in color, attenuated anteriorly. Mouth opens into a pharyngeal capsule, which is marked in front by an annulation. Esophagus is thick and muscular, and gradually increases in diameter posteriorly. Intestine rather sinuous, with thin, delicate walls, dark in color.

MALE 6 to 8 mm. long (according to Siebold). Body ends abruptly behind; membranous fanlike bursa with six rays, of which the middle rays are the most marked; two spicules of equal length. Testicle a single very wide tube, sinuous anteriorly.

FEMALE 6 to 13 mm. long by 0.5 to 11 mm. thick. Body becomes gradually thicker in the anterior third, then continues of equal thickness to the posterior extremity. Tail with slender conical tip. Cuticle finely striate. Esophagus equal to one-fifteenth of the body length. Ovaries two, widening into capacious uteri, which unite to form a vagina of about 2 mm. in length. The latter gradually decreases in diameter toward the vulva, a large, prominent opening, with salient lips, situated slightly posterior to the middle of the body.

Habitat.—In the nasal and orbital cavities of *Larus ridibundus*, *Larus fuscus*, and *Larus* sp.

The above description is taken from Ransom's paper and is a compilation of all the available data. It is unfortunate that so little is known of the type species of this genus.

Cyathostoma americana n. sp. (pl. 1, fig. 6, 7; pl. 3, fig. 21, 28).

MALE 12 mm. long, cylindrical. anterior fifth somewhat attenuate; Buccal capsule about as wide as deep, internal transverse diameter 185 μ; depth of the chitinous portion 170 μ; wall of capsule 25 μ thick. Buccal teeth six or seven in number, subacutely triangular in shape, occupying the entire floor of the capsule; teeth not continued up the sides of the capsule in the form of ridges. The largest (lateral) teeth are about 57 μ high. Head papillae six in number, all essentially similar, arranged in a circle, the plane of which lies 60 μ behind the anterior extremity. Each papilla appears as a rounded knob, 10 μ in diameter. Esophagus 730 μ long, clavate, 74 μ thick at the anterior end, 130 μ thick just before the esophago-intestinal valve, nerve ring at the middle of the length, cervical papilla at about posterior seventh of esophagus, excretory pore just before the beginning of the intestine. Entire cuticle of worm smooth. without transverse striations.

Caudal bursa well developed, when spread 750 μ in transverse diameter; ventral rays similar and approximate; externo-lateral shorter than other lateral rays and with a prominent ventral hump; medio-lateral and postero-lateral rays similar and approximate; externo-dorsal ray more slender than but equal in length to the externo-lateral ray, arising at the base of the dorsal ray. Dorsal ray divided near its extremity into two simple branches. Spicules filiform, equal, from 470 to 490 μ long, each with a finely striated wing; spicules united at their tips. Gubernaculum present, 67μ long.

FEMALE up to 30 mm. long, form similar to that of male. Buccal capsule much broader than deep; internal transverse diameter 370 μ; depth of chitinous portion 280 μ; wall of capsule 20 μ thick. Buccal teeth shorter and blunter than in male; largest tooth 60 μ high. Head papillae as in male; slightly more anterior in location. Esophagus 960 μ long, clavate, 130 μ thick at anterior end, 250 μ thick just before the esophago-intestinal valve; nerve ring at middle of length of esophagus; cervical papillae opposite the thickest portion of the esophagus; excretory pore near esophago-intestinal valve. Vulva just before the middle of the body; lips prominent. Anus just before the slender caudal appendage. Eggs 72 μ $\times$ 42 μ, thin shelled, with a small operculum at one pole.

Host.—*Buteo borealis.*

Location.—In posterior thoracic air sacs.

Geographic distribution.—Fairfax County, Virginia, U. S. A.

Type and allotype.—U. S. N. M., Helm. Coll. No. 25969; paratypes No. 25970. One pair deposited in the British Museum of Natural History.

Cyathostoma coscorobae n. sp. (pl. 4, fig. 43, 45, 46).

Syngamus trachealis Wied. (pars), Fox, 1923, Disease in captive wild mammals and birds, p. 650.

MALE 5.5 mm. (estimated) long, anterior portion attenuate. Buccal capsule with straight sides, its lumen 83 μ in diameter, 100 μ deep, lateral walls 13 μ thick. Buccal teeth six in number, alternate teeth about twice size of others, large teeth 33 μ high. Head papillae, as far as could be determined, as in *C. americana*. Esophagus about 700 μ long, greatest width just before the esophago-intestinal valve, where it is 150 μ in diameter. Nerve ring just before the middle of the length of esophagus. Cervical papillae and excretory pore not seen. Cuticle with an exceedingly fine transverse striation which is evident only near ruptured portions and which may be the result of the rupture.

Caudal bursa well developed; when spread 720 μ in transverse diameter. Ventral rays similar, slender, and approximate, about 105 μ long; lateral rays parallel and continuous throughout their length; externo-lateral ray the shortest (135 μ long); medio-lateral and postero-lateral rays 150 μ and 210 μ long, respectively; externo-dorsal ray 165 μ long, arising near the base of the main trunk of the dorsal ray, which is 150 μ to its bifurication; branches of dorsal ray sinuous, each with three terminations, as in *C. bronchialis*. Spicules filiform, from 660 μ to 720 μ long, each with a finely striated wing; spicules united at tips. Gubernaculum present, 92 μ long.

FEMALE about 20 mm. long. Buccal capsule trapezoidal in optical section, 210 μ in depth, its walls 30 μ thick, diameter of the lumen at base 225 μ, at apex 285 μ. Teeth six in number, proportionately lower than in male, the highest teeth 60 μ. Esophagus 900 μ long, its greatest diameter 255 μ just before the esophago-intestinal valve; nerve ring at the anterior, two-fifths of the length of the esophagus. Cervical papillae and excretory pore not seen. Vulva at about the middle of the body length; vagina very short; uteri divergent. Tail acute; anus 225 μ before tip. Eggs oval, 80 μ long by 50 μ in transverse diameter, with a minute operculum at the slightly smaller end.

Host.—*Coscoroba coscoroba* (Coscoroba swan).

Location.—Trachea.

Geographic distribution.—South America (from captive bird in Philadelphia Zoölogical Park).

Type and allotype.—U. S. N. M., Helm. Coll. No. 26128; paratypes in collection of the Zoölogical Society of Philadelphia.

The specimens upon which the above description is based were collected by Herbert Fox and his associates from a Coscoroba swan which died July 26, 1919. The specimens appear to have been left for some time in water with the result that all have ruptured. Because of this, it has been impossible to make accurate measurements of the total length or of the position of the vulva.

CYATHOSTOMA SP.

In the collection of worms received from Philadelphia there are three female Cyathostoma sp. from the blue-winged teal (*Querquedula querquedula discors*). One of the three specimens is in condition for study and belongs to a species of the second group, in which the vulva is at or near the middle. There is nothing to separate the species from *C. lari* Blanch. except that the hosts are widely separated phylogenetically.

NOTE

Since the completion of the present paper, L. Gedoelst [3] has described a new Syngamus, *S. hippopotami*, from the hippopotamus, collected at Nyangwa, Belgian Congo. The worms, judging from the photographic illustration are of the *laryngeus* type; that is, the posterior half of the female is markedly narrower than the anterior half. If this supposition is correct, the species need only be compared with *S. laryngeus* Raill. *Syngamus hippopotami* Gdlst. is a much larger species, the females ranging from 24 to 34 mm. The spicules of the male are smaller and are of two sizes, the shorter being from 12–18 μ, the larger from 18–24 μ. The dorsal ray is bifurcate clear to the base instead of being single, and occasionally each branch bears a small lateral subray. In these, as well as in other characters, the species is quite distinct from *S. laryngeus*. In comparison with species other than *S. laryngeus*, *S. hippopotami* is readily distinguished by the extremely small spicules.

[3] GEDOELST, L. UN SYNGAME PARASITE DE L'HIPPOPOTAME. Ann. Par. 2: 307–311, illus. 1924.

COOPERIA BISONIS, A NEW NEMATODE FROM THE BUFFALO [1]

By Eloise B. Cram

Zoological Division, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

In material collected during post-mortem examinations of buffalo at the National Buffalo Park, Wainwright, Canada, in January and February, 1923, by S. Hadwen and A. E. Cameron, and sent to the Bureau of Animal Industry, U. S. Department of Agriculture, there were numerous specimens of Cooperia which proved to be a new species. It is to this nematode that Cameron [2] refers in his "Notes on Buffalo" and which, through some lapsus, was designated *Haemonchus ostertagi*. The present writer, however, does not find that any of the specimens attain the length described by him—14 mm. for the female. Cameron notes as the pathological picture an inflammation of the mucous membrane of the fourth stomach and duodenum of the buffalo.

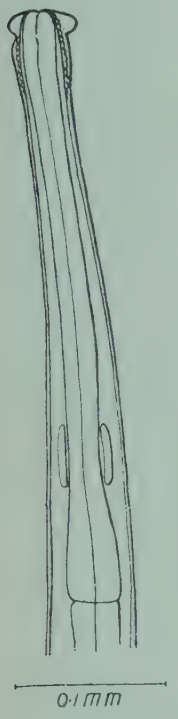


FIG. 1.—*Cooperia bisonis*. Anterior extremity

SPECIFIC DIAGNOSIS

Cooperia: Cuticle of head (fig. 1) markedly expanded, followed by a sharp constriction and then a second flaring out for a short distance. Cuticle shows deep transverse striations throughout dilated region. The nerve ring surrounds the esophagus at a point about three-fourths of the length of the esophagus from its anterior end.

MALE.—The male is 7.2 to 7.7 mm. long by 175 to 190 μ in maximum width just anterior to the bursa. Head 37 μ wide, narrowing down to 33 μ posterior to the cuticular dilation. Esophagus 357 to 382 μ long by 20 μ in diameter throughout about three-fourths of its length, increasing to 33 μ in maximum diameter near the posterior end. Free margins of bursa delicately striated. Lateral lobes of bursa (figs. 2 and 3) curved inward; median lobe divided in two by a small median incision. The externo-dorsal ray is the most slender of the paired rays, the others thicker in the following order: The ventro-ventral, postero-lateral, medio-lateral, latero-ventral, and lastly the externo-lateral, which is by far the thickest. The total length of the dorsal ray is 249 to 257 μ; the stem (149 to 158 μ long to the point of division) bifurcates to form a U-shaped arch. Each branch soon after its division from the main stem gives off exteriorly a thick, thumblike projection about 50 μ long, the free distal half being bent ventrally.

FIG. 2.—*Cooperia bisonis*. Bursa of male; lateral view

[1] Received for publication June 30, 1924; issued June, 1925.

[2] CAMERON, A. E. NOTES ON BUFFALO: ANATOMY, PHYSIOLOGICAL CONDITIONS AND PARASITES. Vet. Jour. 79: 331–336. 1923.

There is little or no tapering of the terminal dorsal branches; near their extremities they are cleft to give off, exteriorly, delicate finely pointed processes; they themselves end bluntly. The spicules (fig. 4) are 224 to 240 μ long and 19 to 24 μ wide; they are of simple structure. The muscular attachment at their proximal end shows plainly as a balloonlike expansion. The telamon (figs. 5A and B) is very elaborate. On the dorsal surface of the genital cone it consists of a strongly chitinized U-shaped structure, and posterior to this

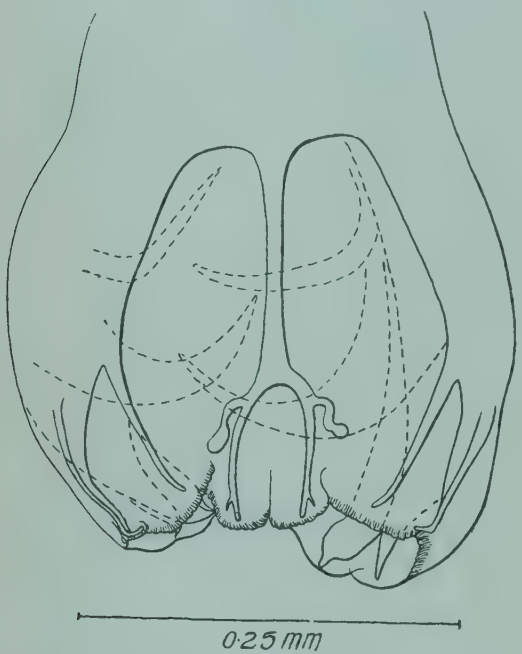


FIG. 3.—*Cooperia bisonis*. Bursa of male; dorsal view

two pairs of papilliform bodies in the lateral fields, and in the median line a process apparently ending in a papilla, extending backward between two chitinized basketlike structures. Masses of granular material extend laterally on both sides of the cone. On the ventral surface there is a prominent chitinized structure consisting of two large, movable, projecting plates. Between them on the posterior surface is a strongly developed rounded body. It is directly below this body, between it and the papilliform process dorsal to it, that the spicules protrude. It is possible that the large, movable projections are clasping organs; the papillae may be sensory in nature; the basketlike structures may serve to support and guide the spicules, and the granular material may act as a cement plug.

FIG. 4.—*Cooperia bisonis*. Spicules

The telamon has not been previously described for Cooperia; it is evidently the most elaborate of any of the telamons studied up to this time. Hall[3] first described this structure in *Hyostrongylus rubidus* and *Ornithostrongylus quadriradiatus*. Lane[4] has noted its character in *Necator congolensis* and Goodey[5] has recently made a detailed study of it in *Oesophagostomum dentatum*. It is evidently present in cylicostomes of the horse, as figures by Kotlán[6] and more recently by Smit and Notosoediro[7] clearly show such supporting structures, although the authors do not call attention to them as telamons. It seems quite probable that this structure will prove to be of value as a definite specific character when it is more widely studied.

FEMALE.—The female is 8 to 9.5 mm. long by 157 to 257 μ in maximum diameter just anterior to the vulva. Head 38 to 41.5 μ wide, narrowing down to 30 to 33 μ posterior to the cuticular dilation. Esophagus 378 to 431 μ in length by 21 μ in smallest diameter, increasing to 33 μ near the posterior end. The vulva (fig. 6) is situated 1.9 to 2.4 mm. from the posterior

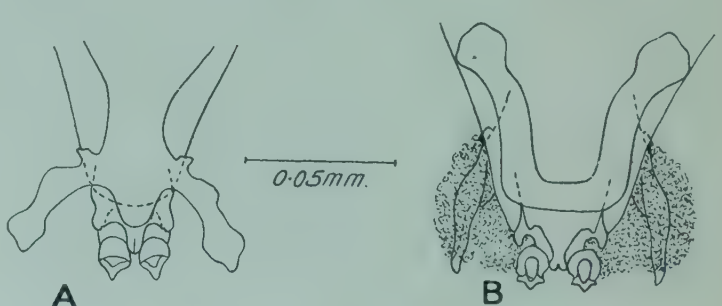


FIG. 5.—*Cooperia bisonis*. Telamon. A, ventral view; B, dorsal view

[3] HALL, M. C. TWO NEW GENERA OF NEMATODES, WITH A NOTE ON A NEGLECTED NEMATODE STRUCTURE. Proc. U. S. Nat. Mus. 59: 541–546, illus. 1921.

[4] LANE, C. SOME STRONGYLATA. PARASITOLOGY 15: 348–364. 1923.

[5] GOODEY, T. THE ANATOMY OF OESOPHAGOSTOMUM DENTATUM (RUD.) A NEMATODE PARASITE OF THE PIG, WITH OBSERVATIONS ON THE STRUCTURE AND BIOLOGY OF THE FREE-LIVING LARVÆ. Jour. Helminthology 2: 1–15. 1924.

[6] KOTLÁN, SÁNDOR. ADATOK A LÓVAKBAN ÉLŐSKÖDÖ STRONGYLIDÁK ISMERETÉHEZ. Néhány új Cylicostomum-faj lovak vastagbeléből. Állát. Lapok 43: 71, 85–86, illus. 1920.

[7] SMIT, H. J., and NOTOSOEDIRO, R. NOG EENIGE STRONGYLIDEN VAN HET PAARD OP JAVA. Nederland. Ind. Bl. Diergeneesk. en Dierenteelt 34: 62–68, 224–232, 446–455, illus., 1923; 35: 29–36, illus. 1924.

end of the body. It has chitinous lips protruding obliquely to the axes of the body; it is covered by a large projecting linguiform process 166 to 177 μ in length, extending backward. The width of the body shows a marked increase just anterior to the vulva, being 157 to 257 μ at this point; posterior to the vulva is a sharp diminution in diameter, it being only 91 to 116 μ wide. The combined length of the muscular ovejectors, including the sphincters, is 532 to 747 μ. The eggs appear strikingly large in proportion to the width of the worm; they are 91 to 99 μ long by 41 to 49 μ wide and are in the morula stage of development when they reach the vulva. The anus (fig. 7) is situated 174 to 190 μ from the posterior extremity. The body gradually narrows posterior to the anus to form a delicately pointed tail bearing at its tip a small bulbous swelling. Toward the extremity the transverse striations of the cuticle are deeper than elsewhere, giving the appearance of pseudo-annulations.

FIG. 6.—*Cooperia bisonis*. Vulva region of female

Host.—*Bison bison.*

Location.—Fourth stomach and duodenum.

Locality.—Wainwright, Alberta, Canada.

Type material.—Bureau of Animal Industry, Helminthological Collection, No. 25516.

Cooperia bisonis is most closely related to *C. oncophora*, but differs from it in showing a more marked thickening of the body in the region of the vulva; in the vulva and anus being somewhat farther from the posterior end of the body; in the large linguiform process overlapping the vulva; in the larger size of the eggs, and in the ventral branches of the dorsal rays originating near the junction of the latter with the stem, rather than from the middle of them. The size of the eggs of *C. bisonis* (91 to 99 μ) exceeds that given in the generic description (60 to 80 μ), so that the latter will have to be modified to include this species.

FIG. 7.—*Cooperia bisonis*. Posterior extremity of female

The points of distinction of the five species of Cooperia found in ruminants are covered in the following key:

1. Total length of dorsal ray 70 to 75 μ; spicules of male less than 200 μ long; vulva of female situated not more than 1.5 mm. from posterior end of body 2
   Total length of dorsal ray 180 μ or more; spicules more then 200 μ long; vulva situated 1.6 mm. or more from posterior end of body 3
2. Branches of dorsal ray curved in shape of a lyre; combined length of muscular ovejectors 375 to 560 μ; chitinous lips of vulva elongated transversely ..... *C. curticei*
   Branches of dorsal ray nearly straight and parallel; combined length of muscular ovejectors 250 to 275 μ; chitinous lips of vulva elongated longitudinally .. *C. punctata*
3. Vulva covered by a large projecting linguiform process; eggs 91 to 99 μ long by 41 to 49 μ wide; spicules 224 to 240 μ long .. *C. bisonis*
   Vulva not covered by a large projecting linguiform process; eggs 60 to 80 μ long by 30 to 36 μ wide; spicules 240 to 300 μ long 4
4. Total length of dorsal ray 220–400 μ; branches of dorsal ray horse-shoe or U-shaped, with cleft tips; combined length of muscular ovejectors 700 μ ..... *C. oncophora*
   Total length of dorsal ray 180 μ; branches of dorsal ray close together, parallel and uncleft; combined length of muscular ovejectors 300 μ ..... *C. pectinata*

SOME PHYSICAL AND CHEMICAL PROPERTIES OF XANTHOPHYLL AND THE PREPARATION OF THE PURE PIGMENT[1]

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INTRODUCTION

Xanthophyll when found in plant materials is associated with carotin. It is present in all green plants along with carotin, but in larger quantity.

Much has been published regarding the carotinoids, but as methods of isolating the pigments have differed somewhat from time to time, it is difficult to say whether xanthophyll was or was not present in any given plant or animal material reported in the literature. It has been definitely established that xanthophyll or its isomer is present in hen eggs and in the body fat of chickens (*7*),[2] and also in yellow corn. Gill (*3*) claimed that a relatively small amount of the pigment in yellow corn is carotin, although Palmer and Kempster (*7*) and Palmer and Eckles (*6*) showed that yellow corn is rich in xanthophyll and that a little of the pigment is found in hemp seed, barley, gluten feed, and red corn.

Some of the work reported in the literature has not been done with sufficient accuracy to warrant a statement regarding the amount of xanthophyll present in certain plant and animal substances. Very often, too, xanthophyll is confused with carotin and vice versa, and sometimes it is known under another name, the name given being entirely misleading, for it is only recently that reliable methods of procedure have been worked out for the separation (*12*) and complete identification of these two pigments.

Even in the more recent literature statements regarding the carotinoids are often difficult to explain. Gill (*3*) states that none of the pigments of yellow corn, mustard, and orange peel are extracted from a petroleum ether solution by 80 to 90 per cent alcohol, although large amounts are absorbed by calcium carbonate. Since none of the pigments are extracted by the alcohol it would appear that xanthophyll is not present, and again, since adsorption by calcium carbonate is large, it appears that the pigment is xanthophyll. Another instance of seemingly contradictory results can be found in the work of Palmer and Eckles (*6, p. 362*), if the standards set by Tswett (*10*) regarding xanthophyll are accepted; the xanthophyll-like constituents of yellow corn were not adsorbed by calcium carbonate from a carbon disulphide or a petroleum ether solution and, on the other hand, the pigments could be completely extracted from a petroleum ether solution by 80 per cent methyl alcohol.

Here are two cases of xanthophyll behavior which do not agree with the generally accepted idea of its behavior. The xanthophyll reported by Gill could not have been xanthophyll, since it was not extractable by alcohol, while that reported by Palmer and Eckles would be xanthophyll, according to the standard set by Willstätter, since it was extractable by 80 per cent alcohol. These two cases of conflicting statements make it quite plain that there is need of work on the Tswett (*10*) adsorption method in connection with the distribution of carotin and xanthophyll between petroleum ether and alcohol. It is very desirable that the different xanthophylls, as described by Tswett, be obtained in crystalline form so that the pure pigments, if they really exist, may be identified.

Palmer and Eckles also have shown how the xanthophyll-like fraction from carrots produces several adsorption bands when the carbon disulphide solution is passed through a calcium carbonate adsorption column. The pigment of Zone I appears entirely unlike the others, for it is completely adsorbed by calcium carbonate from a carbon

[1] Received for publication June 13, 1924; issued June, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 584.

disulphide solution, while the others are not adsorbed but pass through if more carbon disulphide is added. It appears as if several xanthophylls were present.

ISOLATION AND PURIFICATION OF XANTHOPHYLL

In order that the purity of the preparation of xanthophyll used in the work herein described may be unquestioned, and also to aid those who are desirous of obtaining the pure pigment, it is considered advisable to describe very fully the method which has been adapted from those described by Willstätter and Stoll (*12*).

The best source of xanthophyll is dried green leaves. The leaves may be kept for several months and the xanthophyll extracted when desired. Although the leaves of almost any green plant would be suitable, cowpea leaves were used in this investigation. The leaves are carefully spread out to dry, away from sunlight. A large oven with screen trays may be used, or a large screen on which the leaves may be stirred. Room temperature is hardly sufficient, but for drying small quantities it serves very well. Rapid drying is preferable, though great care must be used not to get the temperature too high (above 40° C.), since too high a temperature destroys a large percentage of the pigments present in the leaves. The drying will take from 12 to 24 hours, or a little longer if large quantities of leaves are used.

As soon as the leaves are dried they are placed in a large pebble mill—the mill in use in the Office of Soil Fertility Investigations is about 38 cm. in diameter and 45 cm. in length—and ground 12 to 24 hours. When ground to powder, they are removed from the mill and placed in either glass or tin containers. Since the carotinoid pigments oxidize very readily, the material should be kept in sealed containers. Leaf material may be kept in this manner for several years, and used as desired for the preparation of xanthophyll.

The method here described will be suitable for obtaining 0.5 to 0.8 gm. of pure xanthophyll from 2 kgm. of the dried leaves. A quantity of leaves sufficient to produce this amount of xanthophyll is about all that can be easily handled with ordinary laboratory apparatus, and even then some special apparatus will be desirable.

Pure chlorophyll and carotin may also be prepared (these processes will not be described here) at the same time from the extract by a little more labor, if the worker desires the pigments. The method of preparing pure xanthophyll is essentially that given by Willstätter and Stoll, but certain modifications which are very necessary for good results will be added.

Two kilograms of the leaf powder are placed upon a large porcelain Büchner funnel (25 to 30 cm. in diameter) to which a filter paper has first been fitted. The leaf powder is spread out carefully but not compressed. The funnel has previously been fitted to a 4-liter filter flask which is connected to the vacuum pump. At first 80 per cent acetone is allowed to soak into the dry leaf powder, which is stirred with a spatula or glass rod in order to hasten this part of the process. As soon as the 80 per cent acetone has thoroughly moistened the leaf powder, suction is applied and more acetone is poured on. If the leaves are compressed or suction is applied before the leaf powder is thoroughly moistened with the aqueous acetone, filtration will be greatly retarded. The green acetone extract is sucked off and more acetone added in liter portions until approximately all of the leaf pigments have been extracted. About 6 to 8 liters of 80 per cent acetone will be required for the extraction, which should be completed in from 30 to 60 minutes.

About half of the acetone extract is now poured into a 7-liter separatory funnel and 2 liters of distilled petroleum ether are added. The whole is shaken thoroughly, and about 250 c. c. of distilled water added in order to cause a separation of an aqueous acetone layer, which is drawn off. The remainder of the acetone extract is added to the separatory funnel, shaken, and the acetone layer is again separated by adding another 250 c. c. portion of water. The aqueous acetone solution contains the yellow flavones which are soluble cell sap pigments.

To the petroleum ether solution remaining in the funnel 1,000 c. c. of 80 per cent (by volume) acetone is added and shaken thoroughly. This solution is allowed to stand a minute and run off. The impurities are thus removed and only a very little of the xanthophyll is lost. The acetone is now partially removed by four successive washings, each with 250 c. c. of distilled water. Four washings are sufficient, for if more of the acetone is washed out the xanthophyll will crystallize during subsequent washings with methyl alcohol and the separation of the xanthophyll from the chlorophyll will then become much more difficult.

To separate the xanthophyll from the petroleum-ether acetone solution 1 liter of 80 per cent methyl alcohol is added, the solution is shaken three times, and the yellowish-green methyl alcohol layer is drawn off each time. If the last extract is still quite yellow more extractions will be necessary. The methyl alcohol extracts are combined and placed in a 7-liter separatory funnel or a 5-gallon water bottle. The petroleum ether solution by further treatment may be used for the preparation of chlorophyll and carotin.

The xanthophyll is extracted from the methyl alcohol by mixing the combined xanthophyll extracts with 4 to 6 liters of ether. This solution is shaken thoroughly, and a saturated aqueous sodium chloride solution equal in amount to the methyl alcohol is added. The ether layer containing the xanthophyll and traces of chlorophyll will separate above and the aqueous methyl alcohol layer below. The aqueous methyl alcohol layer is then run off. About 2 liters of ether are added to this aqueous methyl alcohol solution and shaken again; more of the salt solution is added and allowed to stand a half hour, when the ether layer will have separated. The ether extraction is repeated if necessary. If a 5-gallon bottle is used the aqueous methyl alcohol layer can be siphoned off into another bottle and then the ether solution of xanthophyll may be poured into a separatory funnel, where the last traces of methyl alcohol and acetone may be washed from the ether.

The ether solutions of xanthophyll are all combined in a 4 or 6 liter separatory funnel and washed several times with water to remove alcohol and traces of acetone. The yellowish-green ether solution which contains the xanthophyll and some chlorophyll *b* is now dried by shaking with anhydrous sodium sulphate (100 to 200 gm.). After pouring the ether solution from the sodium sulphate it is shaken with 50 c. c. of concentrated methyl alcoholic potash.

Saponification is best carried out at room temperature in a gallon bottle or in a separatory funnel and is done in order to remove chlorophyll *b*, the potash salt of which is soluble in water. The water soluble salt of chlorophyll *b* is now washed from the ether, 100 to 200 c. c. of water being used for each of several washings. The xanthophyll remains in the ether.

The ether solution is dried with anhydrous sodium sulphate, then filtered and evaporated (using diminished pressure toward the last) to about 25 c. c. on a water bath at a temperature not exceeding 50° C. The xanthophyll should not remain in contact with ether long, for experiments reported below show that ether will oxidize the xanthophyll and cause a lower yield. The concentrated ether solution of xanthophyll is mixed with 200 to 300 c. c. of absolute methyl alcohol while the solution is still in the water bath and the ether is removed under reduced pressure. The methyl alcohol solution is filtered through a Büchner funnel with suction while hot to remove impurities and the last traces of ether. The concentrated solution of xanthophyll in methyl alcohol is now allowed to stand in the ice chest for a day or more, when beautiful crystals of xanthophyll will have separated. In order to make the separation complete a little water is added, when the xanthophyll will form crystalline aggregates which are arranged radially. On standing for a day or two, the aggregates gradually change to the usual form of leaf clusters. The yield of xanthophyll when separated from the above mother liquor by filtering and washing with petroleum ether to remove traces of fats, etc., is 0.5 to 0.8 gm., or more. The xanthophyll must be further purified, however, if it is to be used for accurate work.

Xanthophyll takes up ethyl or methyl alcohol in crystallizing and may be freed of this by precipitating from chloroform with petroleum ether. Its further purification is completed as follows: The crystals of xanthophyll, obtained as described above, are carefully dissolved in boiling absolute methyl alcohol. This is done by placing the crystals in the alcohol and bringing to a boil on the water bath for a short time (1 to 5 minutes). This solution is filtered by suction to remove any undissolved crystals. The saturated solution is then placed in the ice box over night or longer. The crystals will separate in much larger plates than before and have an unusually strong surface luster. They are filtered off by suction, washed with low-boiling petroleum ether, recrystallized a third time, and often a fourth or fifth time, from methyl alcohol and then dissolved in chloroform. The chloroform solution is concentrated by suction and warming until xanthophyll begins to separate; the solution is then warmed to bring all of the material into solution and immediately 200 to 400 c. c. of redistilled low-boiling petroleum ether is added slowly. A yellowish

precipitate (the depth of color depends upon several factors) forms almost immediately and can be collected on a hardened filter. The precipitate is washed quickly with pure low-boiling petroleum ether and dried in a vacuum desiccator at once. The drying is only for the purpose of removing the petroleum ether. The melting point should now be 173 to 174° C.

Drying is accomplished in 15 to 20 minutes, and weighings of xanthophyll are made at once for all of the solutions needed. Any xanthophyll remaining may be stored in the ice box in a flask containing petroleum ether, methyl or ethyl alcohol. If more xanthophyll is required later for any purpose the xanthophyll is recrystallized from methyl alcohol and is reprecipitated from chloroform and petroleum ether. In this way a pure product is assured each time. Pure anhydrous ether is the best solvent for xanthophyll, but as deterioration is greatest in this solvent readings must be made at once. If xanthophyll is dissolved in absolute alcohol, difficulty is often experienced in getting the last traces of the pigment into solution. It would seem that pure xanthophyll might be obtained by precipitating several times from chloroform with petroleum ether, as is the case when carotin is purified by dissolving in carbon disulphide and then recrystallizing from petroleum ether. Observations have shown, however, that xanthophyll can not be purified in this manner. To get the purest xanthophyll preparations, spectrophotometer readings show that crystallizations must first be made from methyl alcohol and that the xanthophyll must then be precipitated from chloroform by the addition of petroleum ether. Melting-point determinations are not as good a criterion of purity as are spectrophotometric observations, as data given in a former paper (*8*) show.

CHEMICAL AND PHYSICAL PROPERTIES OF XANTHOPHYLL

SOLUBILITY

Solubility determinations were made, using petroleum ether which fractionates at 50 to 55° C., absolute ethyl alcohol (99.7 to 100 per cent), absolute methyl alcohol, and anhydrous ether which had been specially prepared by washing U. S. P. ether with water four times, distilling over calcium chloride and then allowing to stand over sodium for a week, finally distilling immediately before use.

The xanthophyll used here was prepared fresh in every case as described in the preceding section. Solubility tests were made in a water bath kept at 25° C. and the pigment content was determined by means of the spectrophotometer and the mercury line 435.8 mμ. The stability of xanthophyll in absolute ethyl alcohol, in absolute ether, in U. S. P. ether, and in petroleum ether was also tested in this manner, since the results obtained in quantitative work depend upon the stability of xanthophyll in solution.

The solubility of xanthophyll differs considerably from that of carotin, and this property is utilized in the separation of the two pigments. The solubilities were determined in practically the same manner as were the solubilities of carotin (*9*).

Table I shows the solubility of xanthophyll in petroleum ether. The transmittancies given for all of the solvents are evaluated from graph 11, Figure 2 of a previous paper (*8*), for the quantitative determination of xanthophyll in solution. The determinations when averaged show the solubility to be 9.51 mgm. per liter.

TABLE I.—*Solubility of xanthophyll in petroleum ether (B. P. 50 to 55° C.) at 25° C.*

Experiment No.	Dilution	Time in water bath	Transmittancy	Xanthophyll per liter
	Times	*Hours*		*Mgm.*
1	5	22	0.174	9.10
	5	70	.140	10.15
2	5	42	.174	9.10
	5	56	.184	8.80
3	5	60	.133	10.40

The results for absolute alcohol do not check as well as do those for the other solvents. It would seem that the later experiments reported in Table II were the best, for impurities in xanthophyll tend to increase the amount of material held in solution. The average of the last two experiments has been taken as the solubility (201.5 mgm. per liter) of xanthophyll in absolute ethyl alcohol.

The solubility determinations in absolute methyl alcohol are given in Table III. Only the data for experiments Nos. 2, 3, and 4 have been averaged for ascertaining the solubility in methyl alcohol, as there is some indication that the first experiment gave too high a result. This average shows the solubility of xanthophyll in absolute methyl alcohol to be 134.9 mgm. per liter.

TABLE II.—*Solubility of xanthophyll in absolute ethyl alcohol at 25° C.*

Experiment No.	Dilution	Time in water bath	Transmittancy	Xanthophyll per liter
	Times	*Hours*		*Mgm.*
1	100	16	0.0696	276
	100	47	.0742	271
	100	64	.0822	258
2	100	22	.0846	256
	100	46	.0952	243
	100	94	.1020	237
3	50	42	.0192	206
	50	56	.0214	199.5
4	50	60	.0215	199

TABLE III.—*Solubility of xanthophyll in methyl alcohol at 25° C.*

Experiment No.	Dilution	Time in water bath	Transmittancy	Xanthophyll per liter
	Times	*Hours*		*Mgm.*
1	200	22	0.4420	166
	100	70	.2620	138
2	50	42	.0754	134
	50	56	.0740	135
3	50	60	.0816	129
4	50	60	.0649	141.5

TABLE IV.—*Solubility of xanthophyll in ether at 25° C.*

Experiment No.	Dilution	Time in water bath	Transmittancy	Xanthophyll per liter
	Times	*Hours*		*Mgm.*
1	500	22	0.151	975
	500	46	.161	945
	500	94	.155	965
2	500	42	.174	910
	500	56	.156	965

TABLE V.—*Solubility of xanthophyll and carotin*

Solvent	Milligrams per liter	
	Xanthophyll	Carotin
Petroleum ether [a]	9.5	626.0
Absolute alcohol (ethyl)	201.5	15.5
Absolute alcohol (methyl)	134.9	[b]
Pure ether (anhydrous)	952.0	1,005.0

[a] The boiling point of the petroleum ether used was 50 to 55° C. for xanthophyll and 35 to 50° for carotin.

[b] Nearly insoluble.

Willstätter and Mieg (*11*) state that the oxidation product of xanthophyll is very easily soluble in methyl alcohol. Tests made during the course of this investigation confirm this statement. They further state that the addition of ether to a solution of oxidized xanthophyll in methyl alcohol will cause the oxidized xanthophyll to separate as a white powder. These statements assist materially in understanding the solubility of xanthophyll in the four solvents used as shown in the tables. The tables show that the alcohols would tend to exhibit too great a solubility when impure xanthophyll is used if the impurity is oxidized xanthophyll; and, on the other hand, the solubility in ether would not be greatly affected, for oxidized xanthophyll is very difficultly soluble in ether. Petroleum ether apparently behaves as does ether, for Table I does not show any great difference in solubility in the sample used in experiment 1 and experiment 2.

The average of the determinations (Table IV) shows the solubility of xanthophyll in ether to be 952 mgm. per liter.

In Table V the solubility of xanthophyll and carotin in these solvents is compared.

Willstätter and Stoll (*12*) state that 1 gm. of xanthophyll dissolves in 700 c. c. of boiling, or in 5 liters of cold, methyl alcohol; in ethyl alcohol it dissolves more easily; 1 gm. dissolves in 300 c. c. of boiling ether and it is so insoluble in petroleum ether that the solvent is not even colored.

From Table V it is seen that carotin and xanthophyll have nearly the same solubility in pure ether. At 25° C. xanthophyll is 13 times as soluble as carotin in absolute ethyl alcohol. The greatest difference in solubility of the pigments in the same solvent is observed in petroleum ether, which dissolves 60.5 times as much carotin as it does xanthophyll.

STABILITY

Four solvents were chosen in which to test the keeping qualities of xanthophyll. All of these are or may be used in the isolation of the pigment from plant materials. The solvents used were: Absolute ethyl alcohol, pure ether, U. S. P. ether as it comes in the container, and redistilled petroleum ether (B. P. 50° to 55° C.).

A solution of xanthophyll (0.210 gm. per liter) was made in freshly distilled anhydrous ether, and of this 10 c. c. portions (2.1 mgm.) were carefully evaporated to dryness in vacuum at room temperature, the solvents were added immediately and

the volumes of the absolute ethyl alcohol, ether, and U. S. P. ether solutions were made up to 250 c. c. In the case of the petroleum ether solution it was necessary to bring the pigment into solution with 20 c. c. of absolute alcohol and then the volume was made up to 200 c. c. by the addition of petroleum ether. These solutions of xanthophyll were then stored at a temperature of 10° to 20° C. and determinations, both colorimetric and spectrophotometric, were made on the solutions. The data are given in Table VI.

The solutions were made up on March 31 and were kept in the ice box, except for short periods when the determinations were being made. Table VI and Figure 1 show clearly that xanthophyll oxidizes quite rapidly in ether solutions, the decomposition being about the same whether the ether was freshly distilled or not.

The oxidation of the carotinoids in ether agrees well with the development of ethyl peroxide $(C_2H_5)_2O_3$ in ether as described by Clover (*1*). There probably is a correlation between the development of peroxide and the oxidation of the carotinoids.

Petroleum ether and absolute alcohol solutions kept well, showing practically no oxidation during the time that they were in storage.

The keeping quality of carotin in the various solvents was quite similar to that of xanthophyll. In other words, carotin and xanthophyll rapidly oxidize when kept in ether solutions, while both carotinoids are stable when kept in a cool, dark place either in petroleum ether or in absolute alcohol, absolute alcohol apparently being the medium in which both pigments are most stable.

OXIDATION OF XANTHOPHYLL AND CAROTIN

The rate of oxidation of xanthophyll has been variously reported in the literature. This is also true of carotin. The oxidation of the pure pigments will be discussed first and then the oxidation of the impure pigments, that is, those that are still present in the leaf tissue.

The melting point of the pigments is lowered when the carotinoids oxidize. On account of this fact it was attempted to ascertain the comparative rate of oxidation of the two yellow pigments by determining their melting points at various intervals of time when the powders were exposed to the air at room temperature. The results were entirely unsatisfactory, for pure xan-

TABLE VI.—*Keeping qualities of xanthophyll in solution*

Solvent	Date	Colorimetric data				Spectrophotometric data	
		Colorimeter readings, depth in mm. using Lovibond slides			Colorimeter determinations, solution undiluted, mgm. per liter, average results	Transmittancy after solutions were diluted 5 times	Spectrophotometer determinations, mgm. per liter
		5	10	20			
Absolute alcohol	Apr. 8	3.4	5.3	9.2	7.53	0.202	8.30
Ether	---do	3.3	5.6	9.3	7.44	.212	8.00
U. S. P. ether	---do	3.0	5.2	9.0	7.96	.190	8.60
Petroleum ether	---do	2.6	4.1	7.1	9.66	.148	9.90
Absolute alcohol	Apr. 24	3.2	5.5	8.9	7.73	.198	8.28
Ether	---do	4.3	7.1	12.5	5.83	.277	6.65
U. S. P. ether	---do	3.8	6.2	10.7	6.63	.222	7.75
Petroleum ether	---do	2.7	4.2	7.2	9.40	.146	10.00
Absolute alcohol	May 24	3.1	5.0	7.7	8.40	.210	8.12
Ether	---do	7.8	12.3	17.1	3.63	.457	4.08
U. S. P. ether	---do	5.7	9.1	14.5	4.53	.359	5.30
Petroleum ether	---do	3.0	4.3	7.6	8.90	.148	9.85
Absolute alcohol	July 21	3.8	5.4	8.7	7.40	.207	8.16
Ether	---do				(a)	b .286	1.30
U. S. P. ether	---do				(a)	b .292	1.26
Petroleum ether	---do						
Absolute alcohol	Sept. 11	3.2	5.1	9.0	7.86	.173	9.10
Petroleum ether	---do	3.4	5.3	9.2	7.53	.214	7.96
		3.5	5.7	9.6	7.23	.205	8.20

a Too dilute to estimate.

b Undiluted.

thophyll is obtained in a very finely precipitated form, whereas carotin is in the form of crystals. To begin with, then, the substances are not in the same state of subdivision. Furthermore, when carotin crystals are allowed to oxidize the outer portion of the crystal oxidizes first and, consequently, melts first in a melting point tube. There is a melting point (which apparently varies with the degree of oxidation) for the oxidized portion of the crystal, and there is also a melting point for the unoxidized portion. This was ascertained by viewing the melting point tubes, after heating so as to melt the outer surface of the crystals, under

Of a solution (0.210 gm. per liter) of xanthophyll in pure redistilled ether, 5 c. c. portions (each representing 1.05 mgm. of xanthophyll) were placed in small crystallizing dishes. Half of these were kept in a dark box and the other half were placed upon the same box, where they were exposed only to subdued light. All were kept at room temperature and protected from dust. Each workday morning as long as the experiment was run 2 c. c. of ether for each sample was used to dissolve the pigments so as to reduce the effect of the protective action of the oxidized portion and also to dissolve the crystals which might form. The experi-

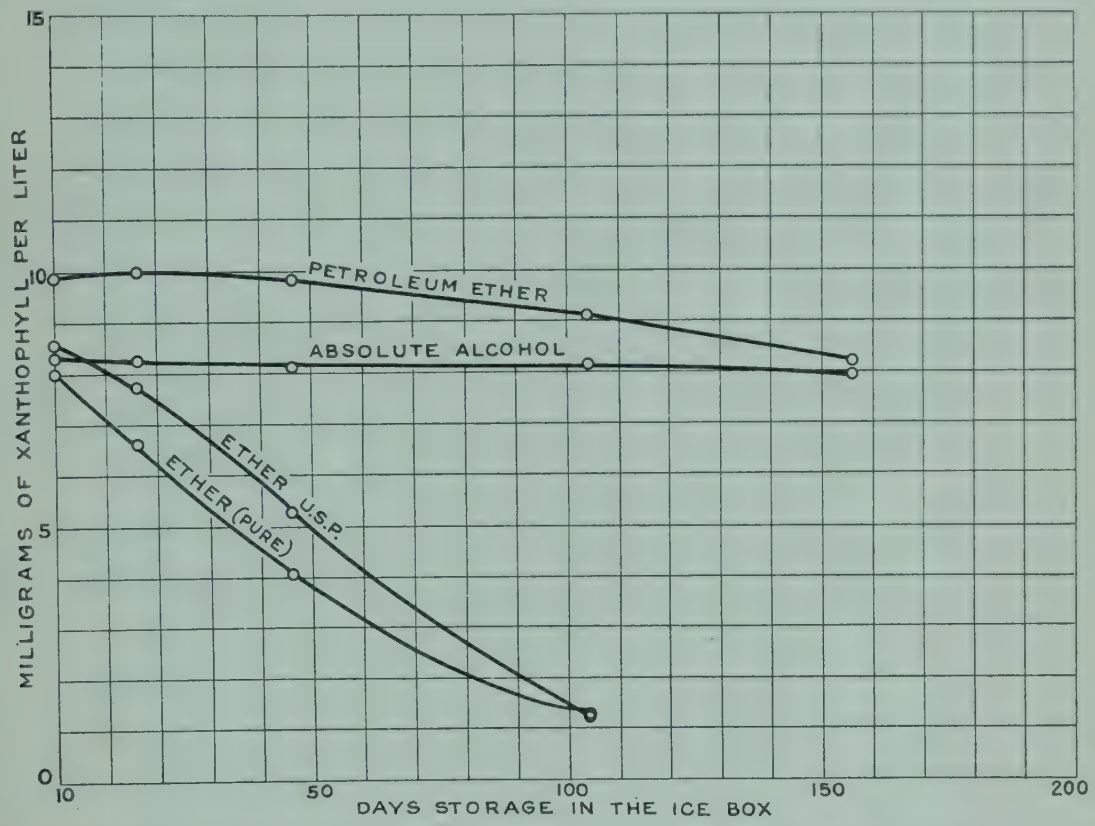

FIG. 1.—Spectrophotometric comparison of the rate of oxidation of xanthophyll in petroleum ether, alcohol, and ether when stored in the ice box at 10° to 20° C.

the microscope. It is obvious that such determinations made on the two pure pigments would be valueless in regard to comparative rates of oxidation. Pure xanthophyll is apparently more readily oxidizable than pure carotin, for all color disappears in the xanthophyll sample before it does in the carotin sample, although this may perhaps be due to the fact that the substances are in different states of subdivision.

Two possible means of determining the relative rates of oxidation of the two pigments will now be considered. The first deals with the pigments in a dry state and the second with the pigments in solution.

ment was begun on March 31, but no quantitative readings were made until April 8. The results of these tests on the rate of oxidation of the pigments are given in Table VII. Samples exposed to light showed a much greater rate of oxidation than those kept in the box at room temperature, protected from light. After 36 days about 25 per cent of the original amount of xanthophyll still remained in the sample in the dark box, while only about 1 per cent remained in the sample exposed to the light, as indicated by the spectrophotometric estimations.

Experiments were conducted with carotin similar to those with xanthophyll. The results are reported in

TABLE VII.—*Oxidation of xanthophyll (in dry form)*

SAMPLE EXPOSED TO LIGHT (ROOM TEMPERATURE)

Days	Date	Spectrophotometric data				Colorimetric data				
		Transmittancy	Volume	Xanthophyll per sample	Xanthophyll unoxidized	Lovibond slide, yellow 5	Lovibond slide, yellow 10	Lovibond slide, yellow 20	Volume	Xanthophyll per sample
			C. c.	*Mgm.*	*Per cent*				*C. c.*	*Mgm.*
0	Mar. 31			1.05	100					1.05
7	Apr. 8	0.324	500	.58	55.2	4.4	7.5	12.3	100	.56
14	Apr. 15	.178	100	.18	17.1	12.2	20.5			[a]
21	Apr. 22	.271	100	.13	12.3	5.0	7.2	13.0	25	.14
28	Apr. 29	.237	25	.037	3.5					[a]
35	May 6	.633	25	.012	1.1					[a]
SAMPLE KEPT IN DARK (ROOM TEMPERATURE)										
0	Mar. 31			1.05	100					1.05
7	Apr. 8	0.216	500	.79	75.2	3.1	5.5	9.1	100	.77
14	Apr. 15	.088	200	.50	47.9	4.5	7.1	11.8	100	.58
21	Apr. 22	.012	200	.44	41.9	3.1	4.9	8.0	50	.42
28	Apr. 29	.027	100	.37	35.2	3.1	5.4	8.5	50	.39
35	May 6	.137	125	.25	23.8	2.4	3.8	6.3	25	.26

[a] Too dilute to estimate.

TABLE VIII.—*Oxidation of carotin (in dry form)*

SAMPLE EXPOSED TO LIGHT (ROOM TEMPERATURE)

Days	Date	Spectrophotometric data				Colorimetric data					
		Transmittancy	Volume	Carotin per sample	Carotin unoxidized	Lovibond slide, yellow 5	Lovibond slide, yellow 10	Lovibond slide, yellow 20	Volume	Carotin per sample	Per cent
			C. c.	*Mgm.*	*Per cent*				*C. c.*	*Mgm.*	
0	May 1	0.443	500	0.44	100	1.3	2.1	3.8	25	0.43	100
7	May 8	.129	125	.28	63.6	2.3	3.5	5.9	25	.27	62.8
14	May 15	.238	125	.19	43.1	3.2	5.2	7.8	25	.19	44.1
21	May 22	.401	125	.12	27.3	5.3	8.3	12.6	25	.11	25.5
31	June 1		125			6.0	9.6	14.7	25	.09	20.9
40	June 10	Completely oxidized.									
SAMPLE KEPT IN DARK (ROOM TEMPERATURE)											
0	May 1	0.443	500	0.44	100	1.3	2.1	3.8	25	0.43	100
7	May 8	.0813	125	.34	78.4	2.0	3.1	4.5	25	.30	69.7
14	May 15	.0982	125	.32	72.7	2.1	3.1	5.2	25	.28	65.1
21	May 22	.145	125	.26	59.0	2.2	3.7	6.4	25	.25	58.1
31	June 1		125			2.3	3.6	6.2	25	.25	58.1
91	Aug. 1	Completely oxidized.									

Table III. The samples (0.44 mgm. of carotin in each evaporating dish) were treated exactly like those for xanthophyll as described above. Both the colorimeter and the spectrophotometer were used in making the determinations. The xanthophyll was dissolved in 95 per cent alcohol and the carotin in high-boiling petroleum ether; on these solutions the colorimetric and spectrophotometric determinations were made. Carotin is seen to be less readily oxidizable than xanthophyll in light as well as in darkness at room temperature.

Carotin has been shown (*9*) to oxidize quite readily in absolute anhydrous ether. Since the ether was not redistilled to remove certain objectionable impurities this experiment has

been repeated; the results are given in Table IX. This experiment was made in order to test the effect of light upon the oxidation of the yellow pigments in ether as well as to test the keeping qualities of the carotinoids in this solvent. The ether in this case was carefully redistilled to remove peroxides; experiments were run using both carotin and xanthophyll. The rate of oxidation of the two pigments in pure ether under exactly the same conditions ought to serve as a good index of their respective rates of oxidation.

The solutions were made up using pure pigments. One sample of each solution was stored in the ice box and one of each was allowed to stand in the sunlight during part of the day. The amount of each pigment in the two solutions was determined colorimetrically and spectrophotometrically on the dates indicated in Table IX.

Carotin is seen to be more stable than xanthophyll in the dark in the ice box, although the reverse is true in sunlight at room temperature. In each case the ether used was exactly the same and, consequently, the results should be absolutely comparable.

in the case of xanthophyll." The purity of all the pigments used by Ewart has been seriously questioned (*5*). Consequently, his work is of doubtful value regarding the yellow pigments reported here.

In researches on the carotin and xanthophyll content of green and yellow leaves, Goerrig (*4*) found that the yellow pigment content of dried leaves (*Fagus silvatica*) was affected considerably by temperature and light during the drying process, as the following data show:

	30° dark	90° dark	Sunlight, room temperature
Carotin	33	25	17
Xanthophyll	59×2	28×2	55×2

At the higher temperature xanthophyll appears to be more sensitive than carotin, while in sunlight carotin is affected more than xanthophyll.

Willstätter and Stoll (*12, p. 105*) found on the examination of a carotin

TABLE IX.—*Oxidation of pure ether solutions of xanthophyll and of carotin*

XANTHOPHYLL

Date	In the dark in the ice box			In sunlight at room temperature			
	Dilution	Transmittancy	Mgm. per liter	Per cent unoxidized	Dilution	Transmittancy	Mgm. per liter	Per cent unoxidized
	Times				*Times*			
May 1	10	0.206	16.5	100	10	0.206	16.5	100
May 11	10	.239	14.9	90.3	2	.065	5.7	34.
May 24	10	.343	10.8	65.4	0	.629	.47	2.
July 21	0	.222	1.5	9.4	Oxidized completely.			

CAROTIN

May 1	5	0.405	4.80	100	5	0.405	4.8	100
May 11	2	.132	4.42	92	0	.364	1.1	22.4
May 24	2	.213	3.34	69.6	Completely oxidized on May 17.			
July 21	0	.375	1.03	21.4				

The oxidation or rate of bleaching of these two carotinoids has been observed by several authors. Ewart (*2, p. 187*) states that "pure samples of xanthophyll oxidize and bleach much more slowly in the light than do impure samples, xanthophyll being less readily oxidizable than chlorophyll and very much less so than carotin," and (*p. 188*) "using watery emulsions of carotin exposed to light and air, the oxidation was much more rapid than

solution (0.0134 gm. in 0.5 liter of petroleum ether containing a little alcohol) stored in a well-stoppered bottle in the dark for three weeks that it had lost no color. A xanthophyll solution (0.0142 gm. in 0.5 liter of ether) quickly bleached; they believed this to be due to impurities in the ether. After two days an ether solution lost 5 per cent and after three weeks 60 per cent of its color. They state (p. 243) that an ether solution of

xanthophyll bleaches with access of air and also very quickly in the dark, much more quickly than carotin. It is not stated whether the carotin was dissolved in ether or alcohol, hence the results may not be comparable.

Observations made have shown that the ether solutions have bleached, while petroleum ether and especially alcohol solutions of pure xanthophyll and pure carotin are apparently stable, as has been shown in this paper. After several weeks the petroleum ether solution of xanthophyll is only slightly bleached.

RELATIVE OXIDATION OF PURE CAROTIN AND PURE XANTHOPHYLL

Ewart states that pure samples of carotin oxidize more easily than do those of pure xanthophyll. Aqueous emulsions of carotin oxidize more rapidly than do those of xanthophyll. Goerrig finds that at high temperature xanthophyll in drying leaves is more sensitive to oxidation than carotin. At room temperature the reverse is true in sunlight. The writer has shown that an ether solution of carotin is more stable at temperatures of from about 10 to 20° C. than is a solution of xanthophyll. An ether solution of carotin is less stable in the sunlight than is a solution of xanthophyll. Carotin in the dry form at room temperature in the light (not direct sunlight) and in the dark is less readily oxidized than is xanthophyll.

In general, it is evident that carotin, in solution or as the pure substance, is more stable than xanthophyll in the dark and more unstable in the light.

Goerrig found that carotin was affected more than xanthophyll when drying leaves were exposed to sunlight. This investigation shows that similar conditions exist in the case of pure carotin and pure xanthophyll pigments when they are in ether solution and exposed to sunlight. Ewart has stated that carotin emulsions bleach more readily than those of xanthophyll in sunlight.

Attention should be called to the fact that the colorimetric and the spectrophotometric readings given above for carotin (Table VIII) do not confirm the following statement made by Palmer and Eckles (*6*): "Spectroscopic bands apparently disappear long before the pigment shows signs of bleaching." In this case the two methods of observation in the work reported here should give far different values (Table VIII) for the amount of carotin present, and the spectrophotometer would give a lower figure. The results in this paper check as closely as could be expected, and so the conclusion is drawn from the work presented that both the spectroscopic and the colorimetric properties of a carotin or a xanthophyll solution vary directly with the pigment content of that solution, which means that the spectroscopic bands disappear only as the solutions show colorimetric signs of bleaching.

SUMMARY

Apparent inconsistencies regarding the behavior of xanthophyll are pointed out in the literature on the yellow pigments.

The isolation and purification of xanthophyll is fully outlined and details of preparation are given.

At 25° C. 9.5 mgm. of xanthophyll dissolve in 1 liter of petroleum ether (B. P. 50 to 55° C.), 201.5 mgm. dissolve in 1 liter of absolute ethyl alcohol, 134.9 mgm. in 1 liter of absolute methyl alcohol, and 952 mgm. in 1 liter of pure anhydrous ether.

Xanthophyll is unstable in ether solutions, is very stable in absolute ethyl alcohol, and is slightly unstable in petroleum ether when kept in the ice box. Carotin is unstable in ether solutions but apparently is perfectly stable in alcohol and petroleum ether.

In the dry state xanthophyll oxidizes more readily than carotin. In solution xanthophyll oxidizes more readily than carotin when kept in the ice box, but when the solutions are kept in the sunlight at room temperature carotin oxidizes more rapidly than xanthophyll. These results are in harmony with those of Goerrig on the oxidation of pigments in drying leaves.

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PHYSIOLOGICAL STUDIES ON CEREALS. III. THE OCCURRENCE OF POLYPEPTIDES AND AMINO ACIDS IN THE UNGERMINATED MAIZE KERNEL[1]

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INTRODUCTION

Up to comparatively recent years the generally accepted idea was that the ungerminated kernel of cereals contains the nitrogen practically only in the form of proteins and nucleoproteins. Having shown in previous papers that the ungerminated kernel of wheat (*Triticum vulgare*) (*8*)[3] and of oats (*Avena sativa*) (*9*) contains in addition to proteins also polypeptides and free amino acids, it was of considerable interest to find out whether or not this holds good for the maize kernel also. This work was prompted not only by the fact that corn (*Zea mays*) is the most important crop in the United States both in acreage and in value (*11*), but also by the consideration that few polypeptides have ever been shown to occur in plant and animal materials and by the further consideration that the nutritive and physiological significance of foodstuffs and feedstuffs will not be fully understood until all their constituents have been determined. It is, therefore, believed that this paper fills a gap in our knowledge concerning the nitrogen compounds met with in the maize kernel.

So far as proteins are concerned, Chittenden and Osborne (*2*) found that the corn kernel contains albumins, globulins, an alcohol-soluble protein (zein), and proteose. The latter, which is found in the extracts of corn meal, may, however, be an artificial product formed by hydrolysis of the above-mentioned proteins. In addition to these proteins, the presence in the maize kernel of glutelin (a protein soluble in dilute alkalis or acids) was later reported by Osborne (*12*). According to Osborne and Clapp (*13*), the proportions in which the proteins occurred in yellow corn meal were as follows: Globulins, albumins, and proteoses, 0.45 per cent; zein (soluble in alcohol), 5 per cent; and glutelin (soluble in alkaline or acid solutions), 3.15 per cent. Similar data have been reported by Johns, Finks, and Paul (*10*). Knowledge is very meager concerning the nonproteins, to say nothing of the complete absence of data with regard to their presence in the different varieties of corn. Owing to the work of Schulze and Castoro (*15*) it is known that the seed of maize contains 0.90 per cent of nonproteins calculated on the basis of the oven-dried seed, or 4.9 per cent if calculated on the total nitrogen content. It is also known from the work of Schulze (*14*) that maize seed contain 0.25 per cent of lecithin. According to Czapek (*3, p. 157*), the proportion of lecithin in the yellow maize seed is 0.25 per cent, while in the white seed it is 0.28 per cent, calculated on the oven-dried seed. In this paper it is shown that the ungerminated corn kernel contains amino acids and polypeptides. While it is true that the proportions in which they occur in the corn kernel are small, they seem nevertheless to be of considerable significance because amino acids are the bricks out of which the great protein structures are built, and for the further reason that the amino acids, being the most reactive material of the cells, are capable of performing important vital functions in the plant and animal organism (*1, p. 61–62*). The polypeptides, too, standing closely to the proteins, no doubt offer the immediate material for the synthesis of proteins. While the maize seed contains great quantities of proteins, the latter are not diffusible. On the other hand, the amino acids, being soluble and diffusible, represent the best material for translocation of the nitrogen to the growing parts of the young seedling, before the proteolytic enzymes in the seed have come into play.

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[2] The writer's thanks are due to J. G. Wangler, junior chemist, for assistance in carrying out the routine work.

[3] Reference is made by number (italic) to "Literature cited," p. 592.

EXPERIMENTAL DATA

MATERIAL USED

Three corn varieties of commercial and agricultural importance were used in the experiments reported in this paper.

The Four County corn variety, which was started with different strains of Silver King, has been bred since 1910 or 1912 by the Four County Grain Improvement Association at Ackley, Iowa, under the direction of the Iowa Agricultural Experiment Station. About 100,000 acres are grown each year in north-central Iowa.

United States selection No. 77, which is a White Dent variety, selected and bred by the United States Department of Agriculture from Woodburn's White Dent, is grown in southern Ohio and Indiana.

United States selection No. 193, which is an 8-rowed yellow flint, selected and developed by the United States Department of Agriculture from Hall Gold Nugget, is grown in the Hudson River Valley in southeastern New York. The corn samples were ground in an electric buhr-mill and passed through a 40-mesh sieve.

DESCRIPTION OF METHODS

(1) The total nitrogen content was estimated according to Kjeldahl's method.

(2) The protein nitrogen was estimated according to Stutzer's method (*17*).

(3) The nitrogen of amino acids was determined according to Sörensen's method (*16*), as applied by the writer elsewhere (*5*, *6*, *7*).

Other methods and their details will subsequently be described in this paper.

From the experimental data presented in Table I it will be seen that whereas the Four County corn is characterized by the highest percentage of total and of protein nitrogen, of dry substance and of ash, Hall Gold Nugget is characterized by the lowest percentage of the corresponding constituents; the figures for United States selection No. 77 are between these two, with the exception of the dry substance, which is lower in United States selection No. 77 variety than in Hall Gold Nugget.

TABLE I.—*Percentage of dry substance, of ash, and of total, protein, and nonprotein nitrogen in the ungerminated maize kernel*

Variety of corn	Where and when grown	Dry substance	Ash	Total nitrogen	Protein nitrogen		Nonprotein nitrogen	
		Air-dry corn	Oven-dried corn	Oven-dried corn	Oven-dried corn	Total nitrogen	Oven-dried corn	Total nitrogen
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Four County corn	At Iowa Agricultural Experiment Station, Ames, Iowa, in 1921.	91.34	1.40	1.74	1.62	95.22	0.081	4.78
	do	91.51	1.39	1.67	1.62	95.31	0.080	4.69
	do	91.41	1.40	1.66	1.64	96.58	0.058	3.42
	do	91.51	1.40	1.71				
Average	do	91.44	1.40	1.70	1.63	95.70	0.073	4.30
United States selection No. 77.	At Piketon, Ohio, in 1919.	90.05	1.32	1.59	1.51	94.96	0.080	5.04
	do	89.95	1.32	1.58	1.52	95.85	0.066	4.15
	do	90.52	1.30	1.59	1.52	95.85	0.066	4.15
	do	89.50	1.33	1.59	1.53	96.14	0.061	3.86
	do		1.34	1.60				
	do		1.33	1.60				
	do		1.32	1.55				
	do			1.61				
Average	do	90.00	1.33	1.59	1.52	95.69	0.068	4.31
Hall Gold Nugget selection No. 193.	At Rhinebeck, N. Y., in 1921.	91.40	1.34	1.46	1.40	96.19	0.056	3.81
	do	91.37	1.33	1.45	1.39	95.26	0.069	4.74
	do	91.37	1.33	1.49	1.39	95.46	0.066	4.54
	do	91.38	1.31	1.46	1.39	95.46	0.066	4.54
	do			1.47				
	do			1.49				
	do			1.42				
	do			1.45				
Average	do	91.38	1.33	1.46	1.39	95.59	0.064	4.41

Experiments conducted with the three corn varieties in order to ascertain how best to extract the nitrogen of the corn flour have shown that the nitrogen extracted by water at room temperature in one hour is as great as that obtained in two and four hours, respectively. In this connection it was found, as would be expected, that a cold aqueous extract of the flour is absolutely free from starch and is very convenient to handle, while a hot aqueous extract contains great quantities of starch and is considerably harder to handle. Since the data concerning the extraction of the flour with water are quite uniform, they are omitted here to save space. It may suffice to mention that in the experiments reported subsequently definite amounts of flour with measured quantities of water were agitated by means of a shaking machine for one hour at room temperature, an antiseptic being used to prevent bacterial action.

The extract obtained was freed from starch, proteins and other insoluble substances by filtration, the filtrate concentrated *in vacuo*, any precipitates formed during concentration were removed by centrifugation, the supernatant liquid evaporated to dryness, and the residue extracted with 80 per cent alcohol, filtered and the alcohol distilled off. The residual yellow sirup was dissolved in hot water, heated to boiling, acidified with acetic acid, boiled, and filtered, the filtrate treated with freshly made lead hydroxide and some lead acetate, and again boiled and filtered. This filtrate was now concentrated under reduced pressure to a small volume.

It should be borne in mind, however, that a water-extract of seeds, prepared in the cold, usually contains proteolytic enzymes which may in part hydrolyze the proteins and proteoses present in the maize kernel. For this reason it seemed necessary to carry out parallel experiments under conditions which destroy the enzymes completely, in order definitely to decide whether or not the amino acids and polypeptides are preformed in the maize kernel. Hence weighed quantities of flour were treated in flasks with boiling hot ammonia-free water and kept on the water bath for about half an hour, whereupon their contents were contrifuged. The solid residue was then treated in the same way once more. The hot water-extracts thus obtained were then treated essentially as described above. The concentrated purified extracts obtained were applied to the estimation of the acid amides, amino acids, and polypeptides.

In order to determine the nitrogen of acid amides, the purified extract corresponding to a definite quantity of flour was made up to 100 c. c., of which two portions of 20 c. c., each were oxidized according to Kjeldahl's method to ascertain the amount of nitrogen present. To 50 c. c. of the remaining solution hydrochloric acid was added to a concentration of 20 per cent and boiled for 30 minutes using a reflux condenser. The hydrolysate was evaporated on the water bath to dryness, the residue transferred quantitatively to a Kjeldahl flask and distilled with magnesium oxide, the ammonia thus obtained being titrated with standard acid.

For the estimation of the nitrogen of amino acids and of polypeptides a sufficient quantity of the purified extract, at least twice the amount used for the acid amide determination, was made up to 200 c. c., in two 10 c. c. portions of which the nitrogen was estimated according to the Kjeldahl method. The remaining 180 c. c. were made up to 200 c. c. and divided into 100 c. c. portions *a* and *b*. In portion *a*, freed from carbon dioxide, phosphoric acid, and coloring matter, the amino nitrogen was determined by the formol-titration method (*16*), while portion *b* was employed for the estimation of the peptide nitrogen. Enough hydrochloric acid was added to portion *b* to make a 20 per cent solution and boiled under a reflux condenser for 12 hours, in accordance with the observations of Fischer (*4, p. 53*). The hydrolyzed material was then evaporated on the water bath to dryness, transferred to a Kjeldahl flask, to which magnesium oxide was added and the ammonia expelled by distillation. The residue was then thoroughly extracted with boiling hot ammonia-free water and the filtered extract concentrated to 100 c. c. of which two portions of 20 c. c. each were oxidized according to the Kjeldahl method, while 50 c. c. of the remaining liquid were used for formol-titration. The result obtained here by titration with formaldehyde diminished by the amino nitrogen secured prior *to* the hydrolysis yields the nitrogen of the polypeptides. The data are recorded in Table II.

Examination of Table II shows that the highest proportion of the nitrogen of acid amides and polypeptides is found in Four County corn, while the highest proportion of amino nitrogen is contained in Hall Gold Nugget selection 193, when the latter is referred to the oven-dried kernel and to its total nitrogen. The figures for United States selection 77 fluctuate, some higher and some lower than for

TABLE II.—*Partition of the nonprotein nitrogen in the ungerminated maize kernel*

Variety of corn	Nitrogen of acid amides	Nitrogen of amino acids	Peptide nitrogen	Remarks
Data expressed in percentage of the oven-dried maize kernel:	*Per cent*	*Per cent*	*Per cent*	
Four County corn	0.034	0.044	0.070	Hot-water extract.
United States selection No. 77	.018	.042	.052	Cold-water extract.
Hall Gold Nugget selection No. 193	.021	.052	.035	Hot-water extract.
Do	.022	.050	.039	Cold-water extract.
Data expressed in percentage of the total nitrogen of the maize kernel:				
Four County corn	2.00	2.59	4.12	Hot-water extract.
United States selection No. 77	1.13	2.64	3.27	Cold-water extract.
Hall Gold Nugget selection No. 193	1.44	3.56	2.40	Hot-water extract.
Do	1.51	3.43	2.67	Cold-water extract.
Data expressed in percentage of the water-soluble nitrogen of the maize kernel:				
Four County corn	16.58	21.68	34.36	Hot-water extract.
United States selection No. 77	11.64	28.11	34.11	Cold-water extract.
Hall Gold Nugget selection No. 193	11.15	27.57	18.55	Hot-water extract.
Do	11.32	26.51	20.66	Cold-water extract.

the last named variety. The outstanding feature of Table II, in which each figure ordinarily represents the average of at least four individual estimations, is the fact that each of the three varieties contains acid amides, amino acids, and polypeptides in not inconsiderable quantities. From the circumstance that the results obtained with the hot-water extracts do not essentially differ from the data secured with the cold-water extracts it follows that under the conditions of the work, extraction with cold water for but one hour and rapid evaporation of the extracts *in vacuo*, no noticeable hydrolysis of the proteins under the influence of enzymes took place. It further follows that amino acids and polypeptides are preformed in the corn kernel.

In the experiments described, the purification of the aqueous extracts was effected by means of acetic acid, lead hydroxide, and lead acetate. This treatment removes completely the proteins but does not remove the proteose quantitatively. Since the latter is present in the water extracts of corn, though in very small quantity, the idea suggested itself that the hydrolysis of the polypeptides might be accompanied by the hydrolysis of the proteose, in which case the results reported for the polypeptides might be somewhat too high (see the fourth column of Table II). Hence, it seemed necessary to remove quantitatively the proteose along with the proteins before hydrolysis takes place. This was accomplished by the use of phosphotungstic acid. The dry residue of the alcoholic extract of a known quantity of flour was dissolved in water and made up to a definite volume, usually to 100 c. c. or its multiple. After determining the nitrogen by the Kjeldahl method in a small portion, the bulk of the solution was treated with 5 gm. of sulphuric acid mixed with 30 c. c. of a solution containing 20 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 c. c. In each case it was ascertained that a slight excess of the precipitant was used. The precipitates formed were filtered, after 24 hours, and washed with a solution made up of 2.5 gm. of phosphotungstic acid and 5 gm. of sulphuric acid per 100 c. c. The quantity of nitrogen present in the phosphotungstic precipitate was estimated by the Kjeldahl method. The filtrate from phosphotungstic acid precipitate was treated with calcium hydroxide to slight acidity, then with barium hydroxide to slight alkalinity, whereupon it was saturated with carbon dioxide, the whole heated to boiling, filtered, and thoroughly washed with hot ammonia-free water. The filtrate and washings from the phosphotungstate, sulphate, and carbonate of calcium and barium were evaporated in a vacuum, made up to a definite volume, and the amino nitrogen estimated by formol titration, while the peptide nitrogen was determined, on hydrolysis, as already outlined. The results secured are summarized in Table III.

A glance at Table III shows that the prevalent quantity of nitrogen is present in the phsophotungstic acid precipitate which contains the proteins, proteose, and any basic compounds occurring in maize, while the nitrogen proportions of the polypeptides, amino acids, and acid amides follow in the order named, with the exception of Hall Gold Nugget selection 193, in which the proportion of amino acids is

TABLE III.—*Partition of the nonprotein nitrogen in the ungerminated maize kernel (hot-water extraction and phosphotungstic-acid method)*

Variety of corn	Nitrogen in phosphotungstic precipitate	Nitrogen of acid amides	Nitrogen of amino acids	Peptide nitrogen
Data expressed in percentage of the oven-dried maize kernel:	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Four County corn	0.076	0.032	0.045	0.069
United States selection No. 77	.061	.019	.040	.050
Hall Gold Nugget, selection No. 193	.076	.021	.051	.036
Data expressed in percentage of the total nitrogen of the maize kernel:				
Four County corn	4.47	1.88	2.65	4.06
United States selection No. 77	3.84	1.19	2.52	3.14
Hall Gold Nugget selection No. 193	5.21	1.44	3.49	2.47
Data expressed in percentage of the water-soluble nitrogen of the maize kernel:				
Four County corn	37.35	15.61	21.97	34.07
United States selection No. 77	40.11	12.18	26.67	32.79
Hall Gold Nugget selection No. 193	39.57	11.20	26.95	19.17

higher than that of the polypeptides. From the fact that the percentage of acid amides, amino acids, and polypeptides obtained by the phosphotungstic acid method is not essentially different from the results secured by the first method (without phosphotungstic acid treatment), it follows that under the conditions outlined the amount of proteose in the aqueous extract of the maize kernel is quite insignificant.

It seemed of certain interest to compare the results at hand with those found for the wheat kernel. The average figures of Table IV were calculated from the Tables II, III, and IV of the paper on wheat (*8*).

TABLE IV.—*Partition of the nonprotein nitrogen in the ungerminated wheat kernel.*

Variety of wheat	Nitrogen of acid amides	Nitrogen of amino acids	Peptide nitrogen
Data expressed in percentage of the oven-dried wheat kernel:	*Per cent*	*Per cent*	*Per cent*
Fultz	0.026	0.032	0.084
Kanred	.053	.067	.111
Kubanka	.052	.041	.155
Marquis	.058	.054	.151
Data expressed in percentage of the total nitrogen of the wheat kernel:			
Fultz	1.46	1.77	4.67
Kanred	1.88	2.35	3.89
Kubanka	1.72	1.35	3.13
Marquis	1.91	1.77	4.98
Data expressed in percentage of the water-soluble nitrogen of the wheat kernel:			
Fultz	8.76	10.66	28.09
Kanred	12.99	16.25	26.86
Kubanka	12.61	9.91	37.76
Marquis	12.33	11.46	32.20

When Tables III and IV are examined it is readily seen that, with but few exceptions, the nitrogen of amino acids is higher in the maize kernel than in the wheat kernel, while the reverse is true of the peptide nitrogen. As to nitrogen of acid amides the figures are rather fluctuating, the total difference between the maize and the wheat kernel being not very considerable.

CONCLUSIONS

Polypeptides and free amino acids, which have been shown in this paper to be present in the ungerminated maize kernel, are performed in it.

The amino nitrogen in the varieties Four County corn, United States selection 77, and Hall Gold Nugget selection 193, makes up, respectively, 0.045, 0.040, and 0.051 per cent calculated on the basis of the oven-dried kernel, and 2.65, 2.52, and 3.49 per cent calculated on the basis of the total nitrogen.

The peptide nitrogen in the varieties Four County corn, United States selection 77, and Hall Gold Nugget selection 193 makes up, respectively, 0.069, 0.050, and 0.036 per cent calculated to the oven-dried kernel, and 4.06, 3.14, and 2.47 per cent calculated to its total nitrogen.

The proportions of acid amide nitrogen in the ungerminated kernel of the varieties Four County corn, United States selection 77, and Hall Gold Nugget selection 193 are, respectively, 0.032, 0.019, and 0.021 per cent calculated on the oven-dried kernel, and 1.88, 1.19, and 1.44 per cent calculated on its total nitrogen.

The varieties Four County corn and Hall Gold Nugget selection 193 are, respectively, characterized by the highest and the lowest percentage of their total nitrogen, as well as of their protein nitrogen.

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RELATION OF WEATHER CONDITIONS TO THE SPREAD OF WHITE PINE BLISTER RUST IN THE PACIFIC NORTHWEST[1]

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INTRODUCTION

Weather conditions to a large extent determine the spread and severity of fungous diseases of plants. White pine blister rust is no exception to the rule. The direction and rapidity of spore distribution over long distances depend upon winds. Favorable moisture and temperature conditions are necessary for germination of the spores and consequent infection of the host plants.

Moisture conditions are more variable than temperature and seem to determine the amount of infection which may occur. If moisture conditions are favorable, temperature conditions also are practically certain to be favorable. Particular attention has, therefore, been given to the correlation between the spread of the rust and the winds and moisture conditions.

The western white pine, *Pinus monticola* Dougl., is practically the only pine to be taken into consideration. There are several species of Ribes in the region under discussion. (See fig. 1.) Some qualifications will be necessary because of differences in both abundance and susceptibility to infection of Ribes in different localities.

It has not been possible to follow the course of the disease from year to year in the Northwest. It has, therefore, been necessary to determine, as nearly as possible, by field studies during the seasons of 1922 and 1923 the year of infection as well as the amount and direction of spread of the rust. This has been done, and it is now possible to determine in a general way how often heavy infections may be expected to occur in places containing both pines and Ribes. It is possible also to make some estimate of the length of time which will be required for the disease to spread by natural means into regions now free from it.

DETERMINATION OF SEASON OF INFECTION

Special efforts have been made to determine the year of infection in all places in which the rust has been studied. In young and vigorous trees it is practically always possible to determine the internode in which infection began. Until recently infections were classified according to the year's internode in which they first appeared. This method was found to be inaccurate. Earlier tabulations in which no reference to the age of the canker was given are not good indicators as the to year of infection.

During the season of 1922 a large number of incipient infections were found in the internodes of 1917, 1918, 1919, and 1920. Table I shows the distribution of 176 of these infections upon a few pines near Bold Point, British Columbia. (For location of infection centers see fig. 2.) There were many Ribes near these pines.

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[2] The writer wishes to express his gratitude to J. S. Boyce, forest pathologist for Washington and Oregon, and to G. B. Posey and S. N. Wyckoff, pathologists in charge of blister-rust control in 1922 and 1923, respectively, for the Western States. Each has been most cordial in his cooperation and has aided in the collection of data in every possible way. Particular credit is due to Harry G. Lachmund, junior pathologist, who most ably and faithfully assisted during the summers of 1922 and 1923 in the collection and preparation of field data. The writer acknowledges the cordial cooperation of the meteorologists and other Government and State officials in the Western States and British Columbia. He is especially indebted to the following: A. T. Davidson, in charge of blister-rust scouting for the Dominion Government, who has furnished valuable data secured by himself and his scouts and who has aided materially in other ways; and F. Napier Denison, superintendent, Gonzales Heights Observatory, Victoria, British Columbia, who furnished all the available meterorological data for British Columbia.

The large majority of the infected internodes produced aecia in 1923 for the first time. As nearly as could be determined, not over 10 or 12 of them failed to produce aecia in April or May, 1923. Upon some of the branches there were so many infections that they coalesced before aecia were produced. On the other hand, some of the 10 or 12 which did not produce aecia may have been 1921 infections, which were beginning to appear in 1923.

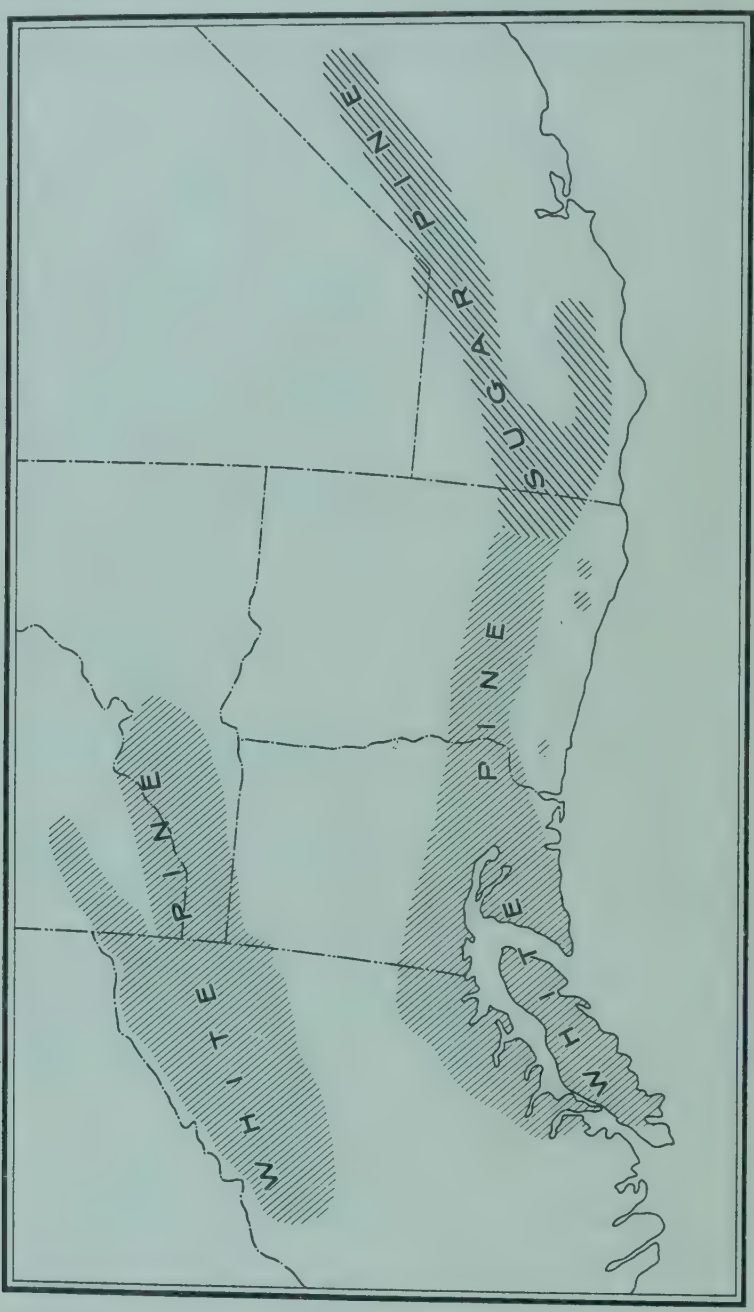

FIG. 1.—Map showing distribution of western white pine (*Pinus monticola*) and sugar pine (*P. lambertiana*) in the western United States and Canada

TABLE I.—*Distribution of 176 infections found in 1922 on pines near Bold Point, B. C.*

Internode of [a]—	Number of infections
1917	5
1918	36
1919	113
1920	22

[a] By "internode of 1917" is meant that part of a stem or branch which began its growth in the growing season of 1917.

It would appear that all or nearly all of these infections occurred in the same year, since they developed practically simultaneously and produced aecia first in 1923. It is very evident that no infections which began in the 1920 internodes could have resulted from exposure earlier than 1920. If those in the internodes of 1917, 1918, or 1919 had occurred earlier than those in the 1920 internodes, they would have been older than the infections in the 1920 internodes and the greater part of them would have produced

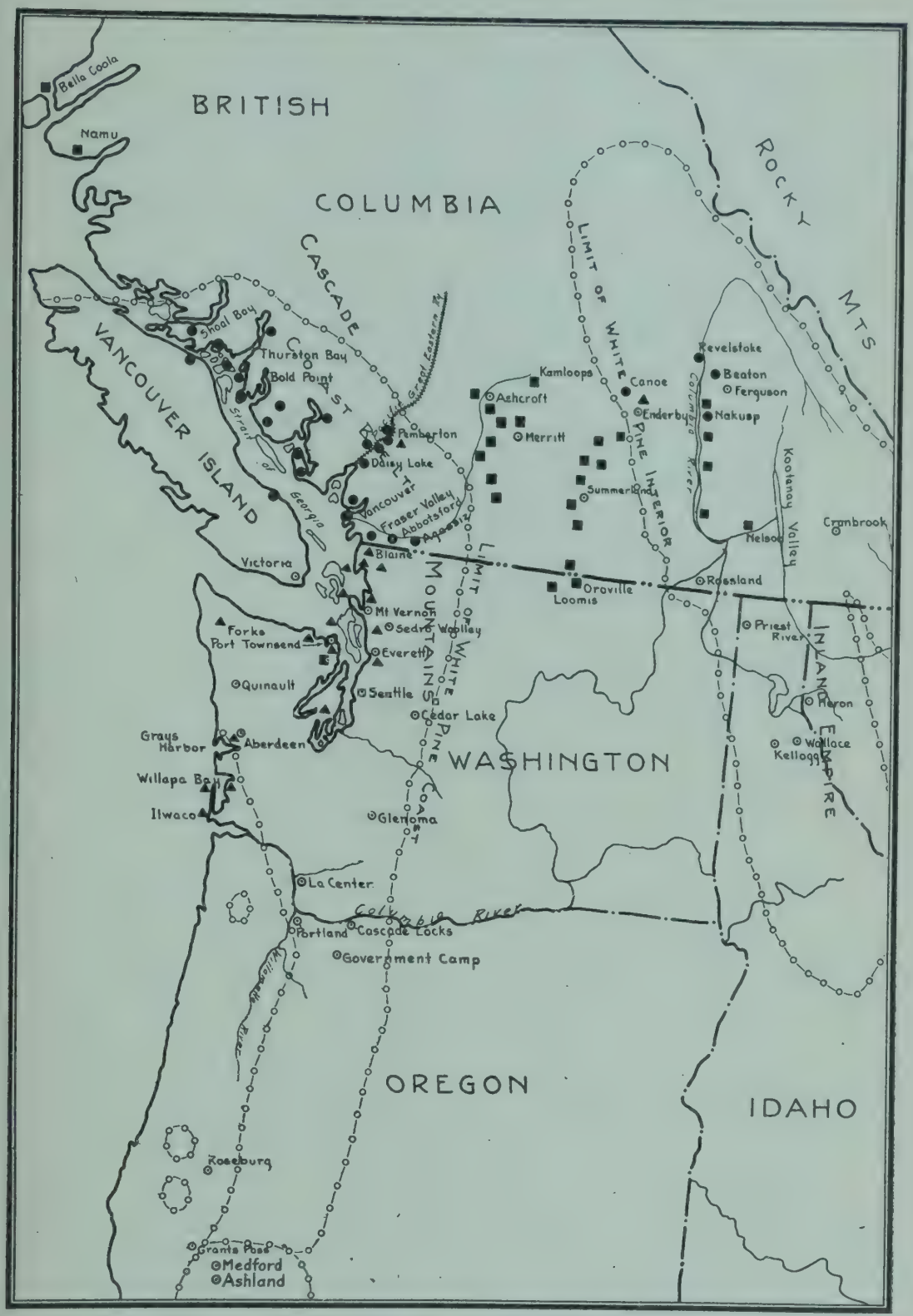

FIG. 2.—Location of infection centers: ●, Pine infection; ▲, 1922 Ribes infection; ■, 1923 Ribes infection

aecia in 1920, 1921, or 1922. On the other hand, if the infections had occurred in 1921, there should have been some fruiting cankers in the 1921 internodes. Several thousand infected branches and stems were examined in 1923. In only one instance was a fruiting canker found in a 1921 internode. It is believed that aecia are usually produced in the third year after infection occurs (*8, p. 25–26*).[3] This is borne out by the writer's field studies both in New York and in the Northwest.

From Table I it is evident that considerable infection occurred in 1920 near Bold Point, British Columbia. Similar observations were made in other localities in the immediate vicinity of older pine infections and Ribes. As field observations were continued through the summer of 1923, it became increasingly apparent that there had been a great deal more infection in 1921 than in 1920. The data given in Table II are typical for conditions found early in July with respect to incipient infections and those which produced aecia in 1923 for the first time.

TABLE II.—*Distribution of 141 infections found early in July, 1923*

Internode of—	Number of infections
1918	13
1919	45
1920	72
1921	11

Nineteen of these produced aecia in 1923 and were probably all infections of 1920. They were distributed as shown in Table III.

TABLE III.—*Distribution of 19 infections, probably of 1920, producing aecia for the first time in 1923*

Internode of—	Number of infections
1918	6
1919	11
1920	2

The 19 infections shown in Table III subtracted from the 141 in Table II leave 122, distributed as shown in Table IV. These are all, or nearly all, infections of 1921.

TABLE IV.—*Distribution of 122 infections found in July, 1923, all, or nearly all, of which are infections of 1921*

Internode of—	Number of infections
1918	7
1919	34
1920	70
1921	11

The totals 19 and 122 (Tables III and IV) show fairly well the relative number of 1920 and 1921 infections apparent by the first week in July. As the season advanced both the actual and the relative number of incipient (1921) infections increased. Table V shows the distribution of 162 incipient infections counted the second week in August.

TABLE V.—*Distribution of 162 incipient infections found during second week of August, 1923*

Internode of—	Number of infections
1918	2
1919	32
1920	97
1921	31

These tables indicate that in any one year infection may occur in the internodes of four successive years.

The western white pine commonly retains its needles four years; that is, during the summer of a given year, as 1921, there will be needles upon the internodes of 1918, 1919, 1920, and 1921. In a favorable season it is probable that infection may occur in any of these needles. Practically all the rust mycelium, however, which might develop in the oldest needles would fail to reach the bark, since those needles would die and fall by the end of that season.

Since in any one year infection may occur in the internodes of three and occasionally four years, it is obviously impossible to determine by the position of a single canker the exact year in which infection occurred. When, however, a large number of cankers of approximately the same stages of development are found distributed through the internodes of three or four succes-

[3] Reference is made by number (italic) to "Literature cited," p. 608.

sive years, it is practically certain that infection resulted from exposure to sporidia in the last of these years.

A great many of the cankers found in 1922 had evidently produced aecia for three years, apparently beginning in 1920. This would indicate infection in 1917. Tabulations made in 1922 included all the infections found in any one place regardless of their stage of development. Table VI shows the distribution of 110 cankers found July 18, 1922, in one limited area near Mile 72, Pacific Great Eastern Railway, British Columbia.

TABLE VI.—*Distribution of 110 cankers found July 18, 1922, near Mile 72, Pacific Great Eastern Railway, British Columbia*

Internode of—	Number of infections
1914	1
1915	8
1916	59
1917	42

The data in this table are fairly typical for the distribution of the older cankers which were found in many places. Infection in 1917 is indicated.

Table VII shows the distribution of 54 cankers as found by A. T. Davidson (*1*) May, 1922, in a swamp at North Vancouver, British Columbia.

TABLE VII.—*Distribution of 54 cankers found May, 1922, in a swamp at North Vancouver, B. C.*

Internode of—	Number of infections
1912 or 1913	5
1915	8
1916	29
1917	12

With the exception of the five in the internodes of 1912 or 1913, these cankers were apparently of the same stage of development. They were probably the result of infection in 1917. The five in the internodes of 1912 or 1913 were several years older than the others. Some of them had started in branches which were dead when found in 1922. It was impossible to determine within two or three years the internode in which infection first occurred. A reexamination of one of the old cankers showed that it might have had its origin in the internode of either 1910 or 1911.

A few of the oldest cankers were found in other places. Two at Daisy Lake and one at Mile 28, Pacific Great Eastern Railway, evidently started in the internodes of 1912 or 1913. One at Thurston Bay, British Columbia, appeared to have started in the 1912 internode; one on Green Mountain, a mile from Bold Point, might have started in either the 1910 or the 1911 internode.

These oldest cankers were not less than 3 nor more than 5 years older than the cankers which seemed to have resulted from infection in 1917. All of them had their origin in the internodes of the years 1910 to 1913, inclusive. Although it is not certain that all began in 1913, any or all of them, from their apparent stage of development and position, might have resulted from infection in 1913. Tabulations of cankers in the immediate vicinity of these oldest cankers do not indicate infection in 1914 or 1915. They do indicate, however, that there was a little infection in 1916, much in 1917, a little in 1918 and 1919, much in 1920, and a great deal in 1921. If the oldest cankers had resulted from infection before 1913, there should have been enough aeciospore production to have caused at least a little infection of Ribes and pine in the immediate vicinity earlier than 1916.

The data presented might seem to indicate that pine infection in any given locality has been largely proportional to the number of aeciospores produced in that locality. This is undoubtedly true in limited areas where an abundance of Ribes is present. It is also true that the greater the amount of aeciospore production the greater the chances for their wide distribution and infection of Ribes. The more important fact, however, is that initial infection of pine in localities where the disease has not previously occurred, and heavy infection near Ribes as well as at some distance from them, have not been proportional to aeciospore production. From the data obtained it is evident that the rust became established in several localities, probably in 1913. No evidence was found to indicate that it was established in any other locality before 1917. There is no reason to believe that there were less aeciospores in 1914, 1915, or 1916 than there were before 1914. As a matter of fact, there were probably many more in 1916 than in any previous year.

In 1917 very heavy infection occurred, not only near the older infections, but at considerable distances from Ribes. At the same time the rust became established in many localities where it had not been present previously.[4] In 1918 and 1919 aeciospore production was probably greater than in any previous year, yet infection was apparently less than in 1917, and the rust became established in few if any places where it was not already present. In 1920 the production of aeciospores was enormously increased, for the infections of 1917 resulted in aecia in 1920. No new centers of infection seem to have been established and heavy infection was confined to the immediate vicinity of fruiting cankers near Ribes.

The field studies of 1923 showed the relatively greater amount of infection in 1921 than in 1920. (See Tables II, IV, and V.) Incipient, or 1921, infections were found in the Fraser Valley near Abbotsford, B. C., and in various places along the Pacific Great Eastern Railway, not only in localities in which no 1920 infections were found, but at much greater distances from the infecting Ribes than any of the 1920 infections. They were found frequently as much as a mile from the infecting Ribes. The 1920 infections, on the other hand, were seldom over 100 yards from the infecting Ribes. The infection of pine in the North Vancouver swamp illustrates very well the frequency of general or widespread infection. In Table VII it is shown that in the North Vancouver swamp there was infection probably in 1913 and again in 1917. Further studies made in August 1923 by H. G. Lachmund [5] showed incipient infections such as those noted in Table V. These indicate a third period of infection in 1921.

From the tabulation given and from numerous field observations, the years of heaviest and most widespread infection appear to have been 1913, 1917, and 1921. As these waves or periods of infection can not be explained on the basis of abundance of aeciospore production, the attempt was made to correlate them with weather conditions.

HISTORY AND DISTRIBUTION OF THE RUST

It has already been shown that considerable infection of pine had occurred in British Columbia not later than 1913. Infection occurred at North Vancouver, at various places along the route of the Pacific Great Eastern Railway, at least 60 miles north of Vancouver, and at Bold Point, Thurston Bay, and Shoal Bay, a distance of 125 miles northwest of Vancouver.[6] There must have been considerable aeciospore production to have caused infection in so many widely separated places.

The only place in which the rust is known to have been present in the Province at that time is Point Grey, near Vancouver. In May, 1922, Davidson (*1*) found on Point Grey, near Vancouver, 180 eastern white pines (*Pinus strobus*), of which 68 were infected with the rust. Some of these had cankers in the growth of 1910. The 180 trees were all that remained of 1,000 which had been imported in 1910 from Ussy, France. Since infected white pines are known to have been shipped from Ussy in 1910 (*7, p. 36*), it is altogether probable that some of the trees imported into Vancouver were already infected. If so, aeciospores should have been produced by 1913, and it is of course entirely possible that they were produced in 1910, 1911, and 1912 also. The plantation on which the infected trees were growing was in an exposed situation upon one of the highest points of Point Grey, where the escaping aeciospores could have been caught easily by the winds and carried in every direction.

It is known that some five-needle pines were imported into Victoria from Europe earlier than 1910. There is no evidence, however, to show that any of them were diseased, nor is there any reason to believe that any pines have been carried into or transplanted in the other places in British Columbia where early infection occurred. Nor is it likely that the rust was carried into the places outside of Vancouver on cultivated black currants, for although these plants are found fre-

[4] LACHMUND, H. G. STUDIES ON WHITE PINE BLISTER RUST IN THE PACIFIC NORTHWEST. Report for 1923. [Unpublished. Typewritten copy in Office of Blister Rust Control, Bureau Plant Industry, U. S. Dept. Agriculture.]

PENNINGTON, L. H. FIELD INVESTIGATIONS OF THE WHITE-PINE BLISTER RUST IN WASHINGTON AND BRITISH COLUMBIA. Report for 1922. [Unpublished. Typewritten copy in Office of Blister Rust Control, Bureau of Plant Industry, U. S. Dept. Agr.]

[5] LACHMUND, H. G. Op. cit.

[6] See map (fig. 2) for localities mentioned.

quently in British Columbia, most of the oldest infections noted above were in places where they were absent. None were found at Thurston Bay and there was no evidence that any had ever been planted in that locality. The oldest infections at Bold Point were in a mountain ravine fully a mile from any black currants. This does not mean that the black currants were not susceptible to the rust, but rather that there was an abundance of susceptible wild Ribes near the infected pines. As a matter of fact, these cultivated black currants apparently caused infection in pines near them in 1917 and again in 1920 and 1921. At Shoal Bay the single old infection and two of more recent date evidently came from old cultivated black currants.

It has been pointed out that there was little or no infection in British Columbia in 1914 or 1915 and very little in 1916. In 1917, however, there seems to have been a great deal and a much wider spread of the rust than in any previous year. It advanced to the east 60 miles as far as Agassiz, 40 miles southeast to Blaine, Wash., north and west 150 miles practically to the limits of the white pine in the coast belt, and apparently eastward for at least 150 miles across the dry belt to Canoe, Revelstoke, and Beaton.

The infections at Agassiz were upon the outer branches of 30-year-old *Pinus strobus*[7] near cultivated black currants. The infected pine near Blaine and in nearly all the other places in the Fraser Valley were found in the vicinity of cultivated black currants.

Fourteen infections were found in 1922 east of the dry belt, 7 at Canoe, 1 at Revelstoke, and 6 at Beaton. They were apparently of the same age and were in the 1916 and 1917 internodes (*1*). Of seven cankers examined by the writer, five were in the 1916 internode and two in 1917 internodes. They were apparently of the same age as those found in the 1915, 1916, and 1917 internodes in the coast region.

No infection was found south of Blaine, although much more intensive scouting was carried on in Washington than in British Columbia. A great deal of the scouting was done by men who worked both in Washington and in British Columbia.

In 1918 and 1919 there was a little infection in the vicinity of the oldest cankers in the coast region.[8] In 1920 there was considerable infection in the immediate vicinity of Ribes near 1917 infections (see Table I). In 1921 there was a much heavier infection than in 1920 (see Tables II, IV, and V).

Very careful search was made during the summer of 1923 in the interior of British Columbia, particularly at Canoe, Revelstoke, and Beaton, to find and destroy all pine infections. Of these 88 are shown in Table VIII.

TABLE VIII.—*Distribution of 88 pine infections found at Canoe, Revelstoke, and Beaton, B. C., during the summer of 1923*

Internode of—	Number of infections at—		
	Canoe	Revel-stoke	Beaton
1917	4	5	1
1918	3	11	1
1919	1	9	9
1920	6	14	10
1921	6	8	----------

In addition to the 88 infections mentioned in Table VIII, 10 were later reported by Davidson (*2*). These with the 14 found in 1922 and the 1 at Nakusp make a total of 113 for the four centers east of the dry belt. These apparently represented three stages in the development of the disease, incipient infections, cankers of one year, and those which had produced aecia for three or four years. This is a very small number upon which to determine the exact years of infection. It has been suggested that the original infection occurred in 1918. From the age of the cankers, however, and their distribution in the branches and stems it seems more probable that infection first occurred in three of the localities in 1917, and that later infection occurred in 1920 and 1921. The single infection found at Nakusp was in the internode of 1919 or 1920. This could not have occurred earlier than 1919, and since it produced aecia in 1923, it probably did not occur later than 1920.

[7] These trees were brought from Ontario in about 1894 or 1895. No evidence was found to indicate that any of them were infected before 1917.

[8] One infection at least occurred in either 1919 or1920 at Nakusp, in the Columbia Valley.

There is no evidence that infection in these widely scattered centers was caused by the introduction of diseased pines or Ribes. No five-needled pines were found, except native *Pinus monticola*.

Since 1912, and particularly during the period of the war, there seems to have been very little transporting or planting of ornamental stock in British Columbia. The infections in pine were found in native trees in the immediate vicinity of old black currant bushes. The bushes at Revelstoke were said to have been brought from eastern Canada in 1898. It would have been a very unusual thing if the rust had been introduced into any one of these places upon pines or Ribes. That it should have been introduced into three or four widely separated places is practically outside the range of possibility. The Canadian scouts were unable to find any evidence that the rust had been introduced upon either pines or Ribes.

Little is known of the distribution of infection upon Ribes previous to 1922, except as it is indicated by the infection of pines. A little scouting was done late in the season of 1921 and some infection found upon cultivated black currants in the Puget Sound region near Port Townsend, Everett, and Mount Vernon, Wash. A great deal of scouting for the rust was done in 1922. Infection was found upon black currants in the Pemberton Meadows and near Mable Lake, B. C., and occasionally upon wild Ribes in many places in the Puget Sound region, near Forks, Grays Harbor, and in the vicinity of Willapa Bay, Wash. The black currant infections in the Willapa Bay region in the extreme southwestern part of Washington were fully 200 miles from the nearest known infection upon white pine and many miles from any 5-needled pines.

In 1923, infection was found upon susceptible wild Ribes in the Puget Sound region as far south as Chimacum, near Port Townsend. The cultivated black currants in this part of the State had been removed in 1922. No infection was discovered upon either the cultivated black currants or the wild Ribes in the Willapa Bay region. On the other hand, cultivated black currants were found infected at 26 places in the dry belt and the Columbia and Kootenay River Valleys in British Columbia.[9] On the coast infection was found at Namu and Bella Coola, 80 and 110 miles, respectively, north of the northern limit of white pine (*1*, *2*).[10]

WINDS IN THE NORTHWEST

The period of aeciospore production was found to be long and to vary considerably in different years. In 1922 aecia began to break open by the first week in May. The heaviest spore dispersal was between about May 15 and June 15. Considerable quantities of fresh aeciospores were found at Vancouver as late as July 5 and some were found in the mountains as late as July 18. In 1923, aecia began to break open the first week in April. The heaviest spore dispersal was between April 15 and May 15. Very few aeciospores were seen as late as June 29.

Possible agents for aeciospore distribution are winds, birds, animals, and man. It is scarcely conceivable that any considerable number of the Ribes infections in Washington or in British Columbia in either 1922 or 1923 could have been caused by spores carried by man. The large majority of the bushes had not been seen by any person who had been in the vicinity of infected pines before those bushes were discovered with infection. There are no other migrating animals which could have carried spores over the wide area in which infected Ribes were found.

Migratory birds move along the coast or north or south in the interior. They do not migrate east and west across the mountains. The species and subspecies of birds east of the Cascade Divide are largely different from those along the coast. It is very unusual to find a bird from the interior west of the Cascade Divide. Ribes infection in Washington was found before the migrating birds had returned from the north. The infections at Namu and Bella Coola are the only ones which by any stretch of the imagination could have been effected by birds. On the other hand, spores carried by winds may account for the infection wherever it has been found upon Ribes.

It is a well-known fact that the prevailing winds of the temperate regions of the earth's surface are from the west or southwest. Cyclones and anticyclones about centers of low or high barometric pressure cause temporary but more or less periodic variation in the direction and velocity of winds. The topography of the earth's surface

[9] See map for localities.

[10] LACHMUND, H. G. STUDIES ON WHITE PINE BLISTER RUST IN THE PACIFIC NORTHWEST. Report for 1923. [Unpublished. Typewritten copy in Office of Blister Rust Control, Bureau of Plant Industry, U. S. Dept. of Agriculture.]

causes local variation in the direction of surface winds. This is particularly marked in a broken country such as British Columbia and the Western States.

There is also considerable variation in the direction of wind in a given season in different years. For example, meteorological records for the northwest coast bear out the statements of close observers that a late spring and a dry summer are accompanied by an unusual aggregate of northerly winds. Tables IX and X show that in May and June the number of northerly winds, that is, those from the northeast, north, or northwest, at Seattle and Vancouver, is very considerable.

TABLE IX.—*Percentage of northerly winds at Seattle, Wash.*

Year	1917	1918	1919	1920	1921	1922	1923
May	28.8	23	30	27	43	27	26
June	36	49	42	34	27	44	41

TABLE X.—*Percentage of northerly winds at Vancouver, B. C.*

Year	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923
May	12	18.2	13.1	19.5	11.5	13	15.1	14.3	20.7	24.8	16.1
June	23	25.1	13.2	17	23.1	27.6	23	33.3	24.5	38.4	24

When taken in connection with Tables IX and X given above, the anemometer records for 1922 show fairly well the amount of wind movement from the different directions at representative stations in the region under consideration. Table XI shows the total number of miles which the wind traveled from each of the eight points during May and June of 1922:

TABLE XI.—*Anemometer records showing the number of miles which the wind traveled from each of eight points during May and June, 1922*

Station	Direction								
	N.	NE.	E.	SE.	S.	SW.	W.	NW.	Total
Vancouver, B. C.:	*Miles*	*Miles*	*Miles*	*Miles*	*Miles*	*Miles*	*Miles*	*Miles*	*Miles*
May	41	211	749	769	312	398	270	706	3,456
June	72	105	443	445	154	310	348	885	2,762
Steveston, B. C.:									
May	70	189	739	1,450	1,172	973	619	1,078	6,290
June	204	22	846	1,235	999	364	582	1,262	5,514
Victoria, B. C.:									
May	724	633	212	387	1,304	4,739	814	27	8,840
June	247	234	61	63	1,966	6,191	241	31	9,034
Summerland, B. C.:									
May	904	303	510	1,168	430	865	716	1,164	6,060
June	1,350	99	83	571	83	1,273	720	2,497	6,676
Seattle, Wash.:									
May	1,103	626	383	825	2,246	1,468	531	307	7,489
June	1,119	523	14	375	1,253	717	661	730	5,392
Portland, Oreg.:									
May	702	199	832	197	561	827	685	2,094	6,097
June	1,082	76	29	127	184	177	185	2,144	4,004
Roseburg, Oreg.:									
May	811	139	28	34	167	346	271	694	2,490
June	1,381	89	31	6	22	60	130	636	2,355
Red Bluff, Calif.:									
May	1,060	36	19	1,045	246	38	291	2,213	4,948
June	323	40	112	1,665	866	50	34	529	3,619
San Francisco, Calif.:									
May	207	81	28	105	77	1,615	4,788	776	7,677
June	-------	32	20	36	23	1,581	5,420	88	7,200

The Victoria and San Francisco stations are in exposed situations and record more of the prevailing winds of the coast. The Vancouver and Roseburg Stations are in more sheltered situations and, consequently, record less of the prevailing winds. The latter do, however, give a good record of local and surface winds.

Anemometer records, as well as the recorded observations of many weather bureau observers, show that the Strait of Georgia, Washington Sound, and Puget Sound form a very favorable channel for the movement of a wide current of air from the northwest down into the interior and southwestern part of Washington. There are periods of one to several days with continuous northerly winds. Between May 26 and June 26, 1922, the Seattle records show that there were three such periods with winds, as follows:

	N.	NE.	E.	SE.	S.	SW.	W.	NW.
May 26–31	502	207	186					60
June 15–18	227	298	3			6	89	143
June 23–26	321	82				3	70	200

The continuity of the surface winds is interrupted by cross currents caused by mountains and valleys below, and by countercurrents in the upper atmosphere. The valley of the Columbia River causes particularly strong currents to the east or west. Pilot balloon records at Camp Lewis, Wash., show that for June, 1922, the direction of the prevailing winds at the surface was northwest and that above 1,500 feet elevation the prevailing direction was southwest. Rarely would it be possible for the same body of air to move from the Strait of Georgia across Washington into Oregon. There was, however, one brief period, June 16 and 17, 1922, when this might have occurred. During these two days there were 502 miles of northerly wind at Seattle, Wash., and 453 miles at Portland, Oreg. The pilot balloon at Camp Lewis showed for June 17 a continuous north wind up to an elevation of 5,000 feet.

In a similar manner the air moves southward over western Oregon, especially along the Willamette Valleys into southern Oregon and northern California. Although there are more intervening valleys and mountains here than in the north, their influence is offset by the relatively greater number of north winds.

Between the Cascades and the Rocky Mountains, wind movements are similar to those west of the Cascades. The anemometer records for Summerland, B. C. (Table XI) may be taken as fairly representative for the valleys which extend north and south in the interior and southeastern part of British Columbia. Observations made twice each day at Nelson, B. C., give the frequency of the winds at that station for 1922 as follows:

Direction	N.	NE.	E.	SE.	S.	SW.	W.	NW.	Calm	Total observations
May	1	7	4	12	1	5	8	17	7	62
June	4	2	2	6	1	2	12	16	15	60

Although there are no anemometer records for northeastern Washington and northern Idaho, the eye observations by meteorologists in that part of the country indicate considerable northerly wind in the north and south valleys. There is probably as much northerly wind at any one place in these valleys as in Puget Sound. They are, however, relatively narrow. The country is more broken, and the daily range of the temperature is greater. These factors all tend to break the continuity of air currents and make it improbable that they can flow continuously in the interior as far as they do along the Strait of Georgia and Puget Sound. It should be noted that Summerland is in the dry country between the white pine belts and that there is a greater amount of northerly wind in the dry belt than in country farther east. The continuity of northerly winds down the Arrow Lake Valley is broken by cross currents at Nelson.

PRECIPITATION IN THE NORTHWEST

Infection of Ribes and probably nearly all infection of pines is dependent upon moisture conditions during the summer months. This statement is based largely upon the writer's observations and experiments upon the rust in the Eastern States. In some localities near the coast fog may be an important factor. Precipitation records, however, have been found to indicate very well the moisture conditions in any given locality, that is, with a few exceptions, the total precipitation for a month indicates fairly well the favorableness of the weather during that month for the development of the rust.

Precipitation records (Table XII) for Vancouver are representative of the moisture conditions for practically all the coast region in which the rust has been found. They also illustrate very well the variation in amount which may be expected in different years at any place in the white pine country (Tables XIII to XVI).

TABLE XII.—*Precipitation, in inches, of rainfall for June, July, and August at Vancouver, B. C.*

Year	June	July	August	Total
1913	3.75	2.02	0.85	6.62
1914	3.58	.42	.75	4.75
1915	.91	.91	.36	2.18
1916	1.34	5.35	.58	7.27
1917	5.40	.48	.93	6.81
1918	1.00	2.20	4.50	7.70
1919	.98	.15	1.15	2.28
1920	3.08	.67	2.91	6.66
1921	3.64	.32	.84	6.80
1922	.17	.02	2.01	2.20
1923	2.08	.52	-------	-------

TABLE XIII.—*Average summer precipitation, in inches, for stations at or near which infection has been found upon pines*

	June	July	August	Total or average for season
Agassiz, B. C.	4.54	2.05	2.55	9.14
Blaine, Wash.	2.12	.88	1.22	4.22
Enderby, B. C.	2.06	1.29	1.21	4.56
Ferguson, B. C.	2.64	2.01	2.06	6.71
Pemberton, B. C.	1.46	.97	1.51	3.94
Revelstoke, B. C.	2.69	2.49	2.49	7.67
Vancouver, B. C.	2.59	1.31	1.74	5.64

TABLE XIV.—*Average summer precipitation, in inches, at stations in the inland region of white pine*

	June	July	August	Total or average for season
Cranbrook, B. C.	1.62	1.51	0.93	4.06
Enderby, B. C.	2.06	1.29	1.21	4.56
Ferguson, B. C.	2.64	2.01	2.06	6.71
Nelson, B. C.	2.44	1.70	1.51	5.65
Revelstoke, B. C.	2.69	2.49	2.49	7.67
Rossland, B. C.	2.33	1.38	1.23	4.94
Priest River, Idaho	1.73	1.13	1.17	4.03
Kellogg, Idaho	1.99	1.10	1.07	4.16
Wallace, Idaho	2.15	1.13	1.21	4.49
Dayson, Mont.	2.35	1.25	1.22	4.82
Fortine, Mont.	2.71	1.52	1.48	5.71
Heron, Mont.	1.89	1.46	1.22	4.57
Polson, Mont.	2.22	1.17	.93	4.32

TABLE XV.—*Average summer precipitation, in inches, at stations in Western Washington, Oregon, and Northern California*

	June	July	August	Total or average for season
Aberdeen, Wash.	3.36	1.17	1.06	5.59
Cedar Lake, Wash.	5.23	2.34	2.48	10.05
Glenoma, Wash.	2.43	1.17	1.50	5.10
La Center, Wash.	2.36	.95	1.06	4.37
Quinault, Wash.	5.29	1.53	2.17	8.99
Sedro Woolley, Wash.	2.68	1.46	1.70	5.84
Cascade Locks, Oreg.	2.39	.80	.92	4.11
Cascadia, Oreg.	3.16	1.04	1.05	5.25
Government Camp, Oreg.	3.28	1.58	1.90	6.76
Prospect, Oreg.	1.44	.66	.32	2.42
Grants Pass, Oreg.	.85	.17	.23	1.25
Jacksonville, Oreg.	.96	.33	.32	1.61
Medford, Oreg.	.84	.43	.13	1.40
Montague, Calif.	.75	.45	.20	1.40
Sisson, Calif.	.62	.16	.30	1.08
Summit, Calif.	.56	.22	.11	.89

All available data show somewhat less summer precipitation in the "Inland Empire" and in the greater part of western Washington than in the coast region of British Columbia. The amount of precipitation for the summer months diminishes southward. In southern Oregon and northern California many seasons have no precipitation during the summer season. Table XVI, precipitation for Summit, Calif., represents the conditions at nearly all places in the sugar-pine country. This shows that rarely is there a summer with precipitation equal to that of the driest of the British Columbia stations at which the rust has been found.

It is to be noted that, as a rule, the summers with the greatest amount of northerly wind have the least precipitation.

TABLE XVI.—*Precipitation, in inches, during June, July, and August, 1871 to 1922, inclusive, at Summit, Calif.*

	June	July	August	Total
1871	0.89	0.00	0.00	0.89
1872	.00	.00	.00	.00
1873	.00	.03	Trace	.03
1874	Trace	.00	.00	
1875	2.55	Trace	.00	2.55
1876	Trace	1.21	.10	1.31
1877	.12	.00	.00	.12
1878	.00	.00	.09	.09
1879	.10	.00	.00	.10
1880	.00	.80	.00	.80
1881	.50	.00	.00	.50
1882	.00	.00	.00	00
1883	.00	.00	.00	.00
1884	4.04	.00	.00	4.04
1885	.80	.00	Trace	.80
1886	.00	.00	.00	.00
1887	1.60	.10	Trace	1.70
1888	3.72	3.51	.28	7.51
1889	.22	.00	.00	.22
1890	.00	.00	.00	.00
1891	.00	.00	.00	.00
1892	.20	.00	.00	.20
1893	.00	.00	.00	.00
1894	.00	.00	.00	.00
1895	.00	.00	.00	.00
1896	.00	.21	.02	.23
1897	0.70	0.00	0.00	0.70
1898	.90	.00	.00	.90
1899	.70	.00	1.00	1.70
1900	.50	.25	Trace	.75
1901	.00	.00	.00	.00
1902	.30	.00	1.00	1.30
1903	Trace	.00	.00	
1904	.05	.04	.03	.12
1905	1.40	Trace	.00	1.40
1906	2.10	Trace	1.00	3.10
1907	2.22	.12	Trace	2.34
1908	.44	.00	.76	1.20
1909	.88	.00	.00	.88
1910	.00	1.16	.00	1.16
1911	.04	.00	.00	.04
1912	.20	.30	.00	.50
1913	.05	2.45	.15	2.65
1914	1.19	.00	Trace	1.19
1915	.00	.55	Trace	.55
1916	.05	.50	Trace	.55
1917	.00	.00	.21	.21
1918	.15	.00	.16	.31
1919	.00	.00	.00	.00
1920	1.60	.00	.67	2.27
1921	.57	.00	.00	.57
1922	.78	.00	.00	.78

DISCUSSION

The prevailing winds and the distribution of precipitation through the summer months have favored the spread of the white pine blister rust in the coast region of British Columbia to the north and northwest, as well as along the valleys to the north and east through the Cascade Mountains. Although aeciospores have been produced in increasing numbers since 1913, at least, and there have been winds sufficient to carry them long distances in every direction, it is apparent that but one season in four has been favorable for any considerable spread of the rust. There was a little infection in the other years. This occurred, however, only in the immediate vicinity of Ribes growing near pines with fruiting cankers.

It is not difficult to understand why very little infection occurred in the years with light summer precipitation, as 1915, with a total of 2.18 inches and 1919 with 2.28 inches, as compared with the average of 5.64 inches, and 6.81 inches for 1917 or 6.80 inches for 1921. On the other hand, it is not easy to see why infection was not greater in other years with a fairly high summer precipitation. In this connection it is necessary to note the requirements for general or heavy infection.

Heavy precipitation in the period of aeciospore production has been observed to reduce greatly the dispersal of spores by washing them out of the aecia. In moist weather many of the aeciospores germinate within the aecia. Their germ tubes then prevent or retard the dispersal of the remaining spores.[11] After the spores are deposited upon Ribes leaves there must be a period with sufficient moisture to permit them to germinate and invade the leaf tissue. After uredospores begin to form there must be occasional moist periods to permit further infection of the leaves if there are to be any considerable number of telia produced. After telia are produced there must be a period favorable for the production of sporidia and the immediate infection of pine needles.

The production of sporidia and the infection of the pine needle seems to be a most critical point in the life history of the rust. York and Snell (*10*) found that a continuous period of 18½ hours with a practically saturated atmosphere is necessary to secure infection of the pine needle. The sporidia are delicate and short-lived. Whatever the combination of circumstances or causes may be, it seems to be a fact that little infection of pine will result in the year with a very dry summer. It is also true that heavy infection does not always occur in years with normal or more than the normal summer precipitation.

The infection in the interior of British Columbia at Canoe, Revelstoke, and Beaton can scarcely be attributed to any other source than wind-borne aeciospores from the coast. The infection at Nakusp may have resulted in the same way or by aeciospores carried down the Columbia Valley

[11] From the author's unpublished manuscript.

from Beaton or Revelstoke. These places are all in the path of the prevailing winds from the coast. There are frequently strong surface winds which blow up through the valley along the Pacific Great Eastern Railway and over the divide into the interior.

The widespread infection of black currants in 1923 at many places in the dry belt, as well as to the east of it, is further evidence of wind dissemination. A glance at the map shows that these places also are all east of the region of heavy pine infection and in line with the prevailing westerly winds from the coast.

The widely distributed infection of Ribes in western Washington in the season of 1922 must have been caused by long-distance spread of aeciospores from British Columbia. This was made possible by the enormous number of spores produced through an unusually long season, from the middle of May until the middle of July, and by the unusual amount of northerly winds at certain periods which came down the Strait of Georgia, through Puget Sound, and spread over the country to the south and west.

All the evidence seems to indicate that long-distance spread of the rust has been caused by wind-borne aeciospores. The only remaining explanation requires long-distance spread by uredospores or a gradual spread and overwintering upon Ribes. Long-distance dissemination of uredospores would be much more remarkable than long distance dissemination of aeciospores. All field studies made by the writer in the East as well as in the West fail to show any considerable spread of the rust by uredospores (*6*). Overwintering upon *Ribes nigrum* has been demonstrated twice under experimental conditions. On the other hand, many experiments have given negative results. Field observations have never shown that the rust has overwintered upon either cultivated or wild Ribes (*8, p. 68–71*). The rust did not survive the winter of 1922–23 upon *R. nigrum* in the Willapa Bay region of southwestern Washington. The bushes on four plantations, all heavily infected in 1922, failed to show any infection in 1923. These were examined in June and again the last week in August. All the other *R. nigrum* in this part of the State were eradicated in 1922. The plants in question were to be destroyed in the autumn of 1923.

It is very improbable that these spores came from local pine infections in Washington. The distribution of the infected Ribes indicated a distant source of spores. If the spores did not come a long distance there must have been many local centers of infection. Thorough scouting, however, failed to show any fruiting cankers in Washington. Many of the infected Ribes, particularly those in the Willapa Bay region, were long distances from any five-needle pines. (See figs. 1 and 2.)

Aeciospores are well adapted for dissemination by air currents. They are dry and powdery and retain their vitality for many days or weeks under adverse conditions. Smut spores are known to be carried long distances by wind (*4*.) Spores of wheat rust have been found over 10,000 feet above the surface of the earth (*9*).[12]

All the species of Ribes found in British Columbia were in a dormant condition and without leaves by the middle of December, 1922, and they so remained until April, 1923. In February, 1923, many plantations of *Ribes nigrum* were examined in Washington and in Oregon as far south as Ashland. All were found to be perfectly dormant, without leaves and with no buds opening. Below Roseburg, Oreg., some wild Ribes were found with a few green leaves of 1922, and buds beginning to open. All the Ribes seen in Washington were in a dormant condition at that time. It has been reported, however, that *R. sanguinium* as far north as Seattle holds a few of its old leaves until the next season's buds open. It appears that the chances for overwintering were very slight even if there had been uredospores upon the old leaves. Many infected *R. nigrum* leaves collected late in October and in November, 1922, were examined. No uredospores, however, could be found upon them.

In the vicinity of Port Townsend, infection was found again in 1923 upon Ribes in the same locality in which it appeared in 1922. In one instance the same plants of *R. bracteosum* and *R. divaricatum* were found with infection in 1922 and in 1923. These were some 9 or 10 miles from the *R. nigrum* which were found infected in 1921. This place is near Puget Sound, less than 80 miles from infected pine in British Columbia. It is altogether probable that these plants as well as many others in favorable situations upon Puget Sound may become infected each year by aeciospores from

[12] Since this paper was submitted for publication, J. A. Larsen (*5*) has called attention to wind dissemination of dust from Oregon and Washington to northern Idaho and Montana.

British Columbia. The probability of overwintering of *Cronartium ribicola* does not seem to be greater in the Northwest than in the Northeastern States.

It is not easy to predict accurately how rapidly the rust will move southward either west of the Cascades or in the "Inland Empire." Its history indicates that its spread upon pine to the south is relatively slow, not over 40 miles since it was introduced into Vancouver in 1910. The fact that north winds accompany dry seasons seems to be an important factor favoring a slower spread of the rust toward the south. On the other hand, *Ribes nigrum*, and to some extent other species of Ribes, may become infected in dry seasons like 1922, and a little pine infection may occur. The volume of aeciospore production is increasing each year in British Columbia. Infection has appeared in the Puget Sound region for at least three years in succession. It is practically certain, therefore, that the rust will become established in western Washington within a few years. When it is once established there, and aeciospores are produced in considerable quantities, it will tend to spread into and through the Cascades to the north and east. Aeciospores may then be carried southward into Oregon and when it becomes established there north winds may carry aeciospores into southern Oregon and northern California.

Although it is scarcely possible that much, if any, infection may occur in the sugar pine region (fig. 1) during the usual dry season, it is very probable that abundant infection may occur in the occasional wet summers such as those of 1888, 1906, and 1913. This possibility is emphasized by the presence of a similar rust, *Cronartium pyriforme*, upon the yellow pine in northern California.

The white pine in the "Inland Empre" is in line with the prevailing winds from the coast. It is also in line with local currents which move southward along the Columbia and Kootenay Valleys. The spread of infection in 1923 to *Ribes nigrum* in the interior of British Columbia and northeastern Washington shows that spores of the rust are practically certain to be carried into the white pine regions of Idaho and Montana. When this happens, infection of pine will depend upon moisture conditions and the presence of susceptible Ribes.

Comparison of the summer precipitation at various stations (Tables XII, XIII, and XIV) in the white pine districts shows less summer rainfall in the interior of British Columbia than upon the coast. It also shows less for Idaho and Montana than for places farther north in British Columbia. The average summer precipitation for these three districts is as follows.[13]

	Inches
Coast region of British Columbia	6.58
Interior of British Columbia	5.93
Idaho and Montana	4.68

These figures indicate, other conditions being equal, that infection of pine should be less severe in the interior than upon the coast of British Columbia. The relatively small amount of infection thus far discovered in eastern British Columbia seems to substantiate this claim (see Table VIII). For the same reason infection should become established more slowly and be less severe in Idaho and Montana than in eastern British Columbia.

The destruction of all infected pine material which has been found in eastern British Columbia should tend to retard the spread of the rust.

It is a significant fact that all the pine infection in eastern British Columbia can be attributed to *Ribes nigrum* and that practically all the infected Ribes found in the dry belt or east of it were this species.

Climatic factors can not be changed materially by man, but the elimination of *Ribes nigrum* from the pine districts of Idaho and Montana should lengthen greatly the time before the rust becomes established in these States.

SUMMARY

The white-pine blister rust has been in British Columbia at least since 1910.

It became widespread by 1913.

It has spread upon the white pine practically to the north and east limits of the coast belt of white pine.

It has spread a relatively short distance to the south.

It has become established at four places in the eastern belt of white pine in British Columbia.

An average of one season in every four years has been favorable for general spread of the disease along the coast of British Columbia.

In the summer of 1922 there was infection of Ribes by aeciospores as far south as Ilwaco, Wash. In 1923 infection was not found south of the vicinity of Port Townsend, Wash.

[13] These figures were obtained by taking an average for the six stations in each district with the greatest summer precipitation. The data for British Columbia is from "Climate of British Columbia," by F. Napier Denison (*3*).

In 1923 infection was found on Ribes at Namu and Bella Coola, 80 and 110 miles, respectively, north of the limit of white pine upon the coast.

Prevailing westerly winds favor aeciospore dispersal from the coast toward the east.

Northerly winds, which favor aeciospore dispersal to the south, are most common in dry seasons, which are unfavorable for pine infection.

West of the Cascades, northerly winds in the period of aeciospore production increase to the southward as far as northern California.

The amount of summer precipitation diminishes southward.

The rust is practically certain to spread southward at a much slower rate than to the north and east.

The spread of the rust to the "Inland Empire" may be greatly retarded by the elimination of *Ribes nigrum* from that region.

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THE CONSTRUCTION OF TAPER CURVES [1]

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INTRODUCTION

Among the first requisites of a forester's equipment in estimating standing timber are accurate and dependable volume tables, applicable to the stands being estimated and the utilization expected. Such tables show, in general, the average volume (in board feet, cubic feet, or other units of measure) of trees of different diameters and heights. It has not proved easy to construct dependable tables of this kind, owing to the difficulty of determining accurately the average volume of trees in the unusual size classes represented by very tall, slender trees and very short, large trees, for it is difficult to discover specimens enough of these classes to furnish the basis of reliable averages.

The first volume tables to be used were constructed simply by scaling a large number of trees of different sizes, averaging the scale of all trees in the same diameter and height class and then harmonizing the values for the different height classes as well as possible by graphic methods. This was the method described in 1906 by Graves (*9, p. 158–163, 166, 167*).[2] It is slow, requires a large mass of data, and the graphic harmonization necessary to make values run smoothly often introduces grave inaccuracy.

In 1915, Barrows (*1, 2*) described a new method, which introduced taper curves, or curves showing the average form of trees, as a step in volume table construction. This was a great improvement over the earlier systems, and has been very generally used since that time. The method was accepted as undoubtedly sound and effective, the results were believed to be entirely satisfactory, and much time and labor were put in on the tedious series of curves necessary in this method. The present writer, however, in making a series of taper curves for lodgepole pine on a slender basis of trees, discovered that very palpable errors had come through the whole series of harmonizations and that the final curves were not dependable. This has led to an analysis of the underlying theory of Barrows's series of recurvings, and to the conclusion that the latter are not as satisfactory as they would appear to be on the surface. It is no new discovery that errors exist in this method of handling taper curves. Barrows himself admits the fundamental error, that taper curves give not the average volume of the trees in any group, but the volume of the tree of average dimensions.

In finding average tree form, diameters are averaged; in the earlier method, volumes proportional to diameters squared were averaged. The error introduced depends upon the range of values in a given class, but, as pointed out by Barrows, it never becomes serious. Bruce (*4*) implies more grave dangers by demonstrating the presence of errors in certain volume tables undoubtedly built up according to Barrows's method. He shows that in these tables the values vary erratically from the volumes of the frustums of cones having the same top diameters and the same breast-high diameters as the trees; a divergence that can not possibly be true if the values pretend to represent true means. The cause of this variation, however, was never determined by Bruce, which leaves an uncertainty as to just what is wrong with taper curves, and whether the difficulty is remediable or not.

NATURE OF UNHARMONIZED TAPER CURVES

In analyzing Barrows's method and the errors inherent in it, consideration must first be given to the basic data from which the taper curves are drawn. These consist of individual tree records giving diameter, usually by regular intervals (often 8 or 16 feet) from the stump to the top of the tree. Height is given to the nearest tenth of a foot; diameter to the nearest tenth of an inch. The size classes which are used as the basis of all computations, and which appear in the final volume tables

[1] Received for publication June 30, 1924; issued June, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 624.

are usually based on 1-inch diameter intervals and 10-foot height intervals. For example, a tree 14.7 inches in diameter at breast height and 82.2 feet tall, belongs in the 15 inch–80 foot class. Trees are usually well distributed as to diameters within a given inch and height class so that the average of all trees falling in such given class is very close to the assigned even diameter. Of course, for each height class there is a mean diameter from which the diameter values fall away on each side roughly in accord with the laws of chance. This curve is long and flat, however, for diameters in any height class, and the distribution of diameters through any single inch class is virtually even, although, of course, theoretically not quite so in any but the model class. Thus, in the 12-inch d. b. h. class, individual trees will range from 11.6 to 12.5 inches d. b. h. Unless there are very few trees indeed in the class, in practice the mean will always fall very close to 12.0 inches. Only occasionally in the highest and lowest diameter classes will this rule fail, as it may indeed by chance in any class represented by a very few trees.

Conditions are not the same, however, in regard to the height. Trees of the same inch class may be tall, short, or medium, and a very wide variation of height values is possible, ranging from two-thirds to one-half of the total height of the trees, in lodgepole pine at least. This variation is very nearly "normal," that is, the heights vary from the central or modal value according to the laws of chance. In case, therefore, the entire sample of data relating to a single inch class is subdivided into five smaller classes on the basis of height, the central class will have evenly balanced values and the actual average will generally represent the true mean of the class. The next class adjacent both above and below will have its values piled up asymmetrically, with a preponderance of values nearer the average height for the inch class. For example, suppose there are five height classes, 100, 90, 80, 70, 60 feet, as shown in Figure 1. The average height of all trees in the central class, 80 feet, will be 80 feet. In the 90-foot class there will tend to be more 86-foot trees than 94, and the average height of the 90-foot class will be lower, perhaps about 88 feet. Conversely, in the 70-foot class values will tend to run high. In the outermost classes this effect is still more pronounced and the average 100-foot tree may be only 96 feet tall, while the average 60-foot tree may be 64. If the tables were to be used only in the region where prepared, this would be of little moment, for in the timber estimate 100-foot trees would again average 96 feet tall and the volume of 96-foot trees would be the correct volume to use. In a region where sites were better, however, trees in the 100-foot class might be 100 feet tall and volumes based on 96-foot trees would be low.

It must be the aim of taper curves and volume tables to give the true middle values of the class represented, or else they will be meaningless. Therefore, the values of the 15 inch–100 foot tree class must be exactly right for trees 15 inches in diameter, and 100 feet tall, and not perhaps for a tree 14.8 inches d. b. h. and 96.7 feet in height. One qualification of any system of volume-table construction must be its ability to take these values that fall a little from the middle of the class they represent and bring them into their proper places.

When diameters taken at intervals up the tree are averaged in each diameter and height class, as is the first step in the preparation of taper curves, there is some question as to the value these averages have and how nearly they truly represent the midtree of the class. This apparently simple operation is not without its complexities.

Sometimes in getting average diameters well toward the top it will be found that certain trees "drop out." For example, if the diameter 78 feet from the base in the 80-foot class is being sought, some trees will "drop out," being less than 78 feet tall and yet over 75 feet tall, and therefore still in the 80-foot class. In averaging they usually are given a value of zero, but it is easily seen that they have a potentially minus value. So, where some of the trees have "dropped out" average diameters are too high. It is well to disregard measurements of diameters at heights where all the trees in the class are not represented, as they lead to error rather than accuracy.

Aside from these mathematical points is the natural fact that the greatest variability in trees is toward the tops. In any given inch and height class the diameter of the trees will vary little more than an inch between maximum and minimum diameters 8 feet above the ground, and perhaps about 2 inches at 16 feet up; while 72 feet up in an 80-foot height class the diameters may range over 5 or 6 inches in variance even in smooth normal trees. Almost every mass of field data contains abnormal trees measured too near swell-

ings, large limbs, forks, etc., which all contribute to wide variability upward. These factors in turn lead to mean diameters containing large probable errors, so much so that when a series of them in consecutive size classes is compared, a great lack of harmony is always found, the amount depending chiefly upon the number of tree measurements used as a basis.

HARMONIZING THE CURVES—BARROWS'S METHOD

Having examined the shortcomings of the original data, the next step is to see how the method of compilation gets around them.

but may vary from the exact even inch; although, as already pointed out, this is unusual, except where only a small number of trees are measured. Average height may also, and very frequently does, fall on one side or another of the exact middle point of the height class as shown in Figure 1. Nowhere does Barrows intimate that this occurs, although he particularly points out the more infrequent case of the average tree failing to fall on the even inch of the proper d. b. h. class.

One fact that helps to prevent the errors in the upper parts of taper curves from becoming extremely serious is the stability of the end point derived from averaging the total heights of the trees.

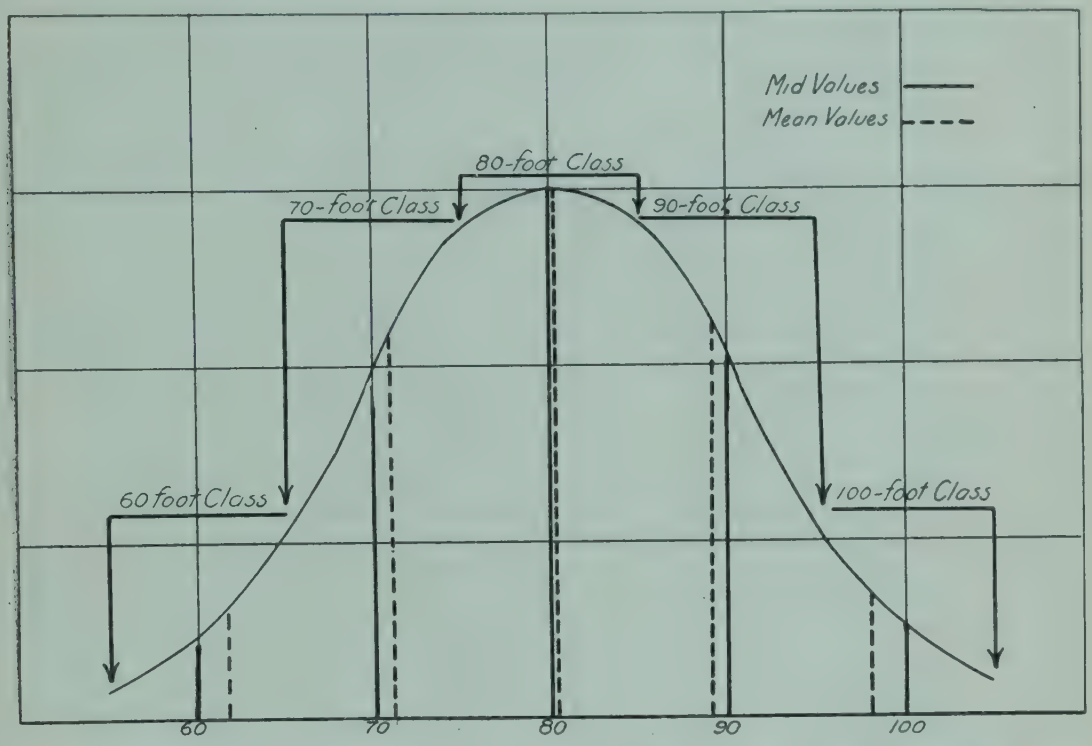

FIG. 1.—Difference between midvalues in various height classes and actual average values due to asymmetrical distribution of values

The conventional method of Barrows consists of several series of curves, of three primary forms shown in Figure 2 in a somewhat simplified form, conical trees and straight lines being used to show more clearly certain points.

The first series of curves, exemplified in Figure 2, A, is drawn through points representing the average diameters at different heights, as compiled from the original tree measurement sheets. All trees of various diameter classes are thrown into one chart, a separate chart being made for each height class. Drawing curves through the plotted points serves to iron out minor irregularities of form and makes the average tree a smooth curve. These curves do not necessarily pass through the exact middle value of each d. b. h. class, At the same time the possibility of errors in the higher parts of all curves presents a considerable problem. Such errors all contribute to irregularities and peculiarities of shape in individual curves which can only be properly straightened out by an efficient method of harmonization.

Figure 2, A, shows the first series of curves simply by the use of straight lines. Accepting Barrows' statement, they are shown failing to pass through the middle value in each inch class (the even inch) at breast height, but at present for simplicity's sake are assumed to converge exactly at the 70-foot point.

The second series of curves is shown in Figure 2, B. In this series of curves the d. b. h. of the tree is made the

abscissa, and the diameter at other points the ordinate. As a rule, values are read at 10-foot intervals up the tree from the curves of series A, and a separate curve is drawn showing the relation of d. b. h. to diameter, 10, 20, 30, and 40 feet above ground level. This series of curves so rounds out the values in Figure 2, A, that, in effect, it takes the lines as shown in Figure 2, A, and turning them upon the point of convergence as a pivot spaces them systematically, placing them upon the correct d. b. h. If the lines in Figure 2, B, are straight, the corrected spacing of the lines in Figure 2, A, will be even; if they are curves, the spacing will be uneven, but the changes will be regular and even (as will occur in cases where form changes with diameter). In practice, lines in Figure 2, B, are nearly straight. Their slope depends upon the distance between the lines in Figure 2, A, at various levels, so there is the greatest slant at the base of the tree where the lines are farthest apart and the least toward the tip.

The spacing between the lines in Figure 2, B, depends upon the slope of the lines in Figure 2, A (taper of the trees). In conical trees they are naturally evenly spaced, as shown in the figure, but in full-boled trees they get farther and farther apart toward the top of the tree, as in such trees rate of taper increases upward. In practical work, the points determining these lines usually fail considerably of falling into good lines, so that in drawing an average curve errors are very likely to be made in giving end values with a poor basis more weight than they deserve, consequently tilting the lines. Marked tilting in any single line will be noticed on inspection of the finished graph, however, unless all the lines are tilted in the same direction (which unfortunately is quite possible). The effect is equivalent to making the lines in Figure 2, A, evenly spaced, but either too far apart or not far enough. The spacing may be different in different height classes, as these are curved entirely independently.

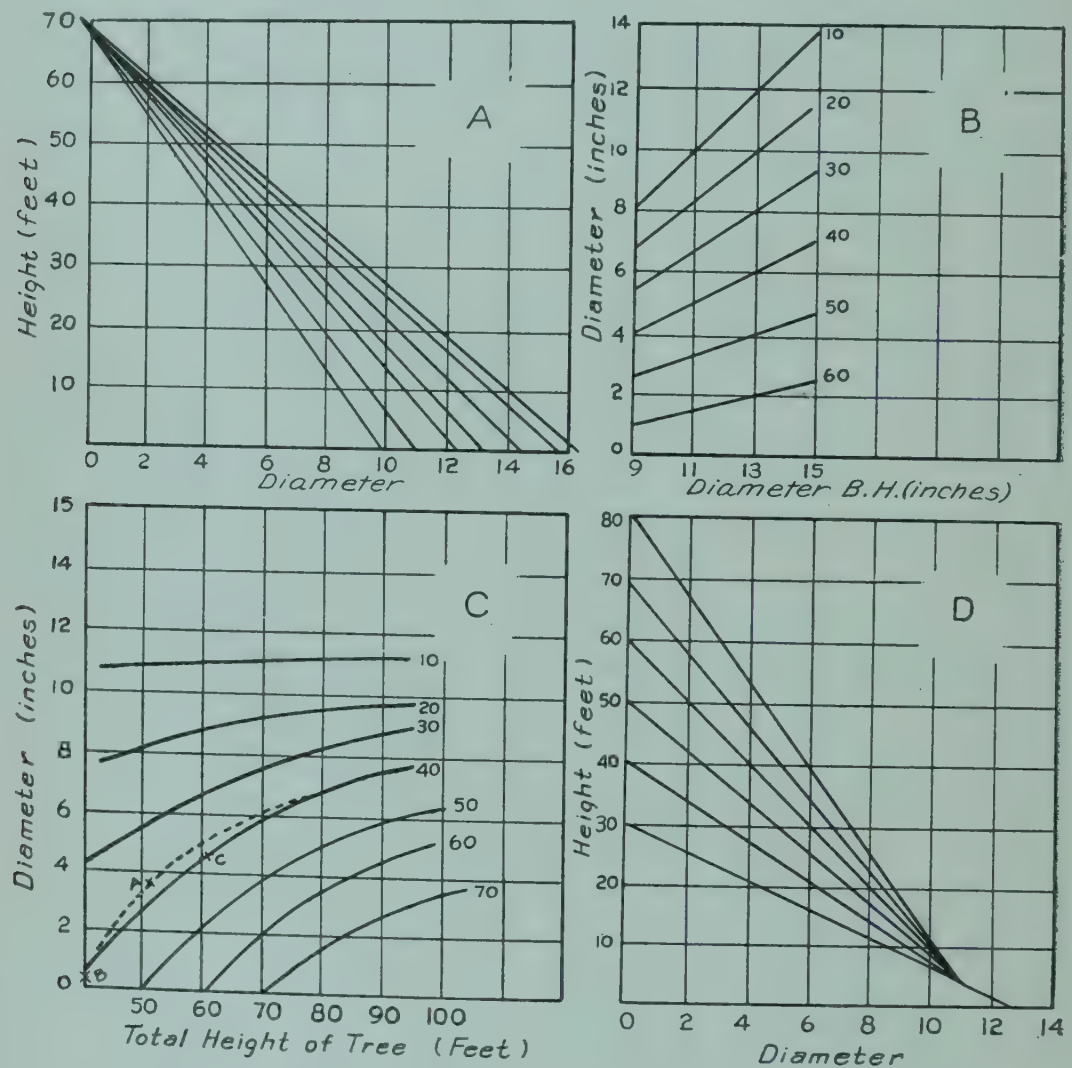

FIG. 2.—The main steps in graphic harmonization of taper curves

The third system of curves is aimed to harmonize values in the same d. b. h. class, but in different height classes. There are never many height classes in a single d. b. h. class, for the variation in the height of trees of a given diameter never amounts to many ten-foot intervals (the usual classification). The curves are, therefore, short and more open to error in direction than long curves. Their form is illustrated in Figure 2, C, and their function is similar to curves of the second series as shown in Figure 2, B, except that d. b. h. is constant instead of the height of the tree, and separate charts are drawn for each d. b. h. class. Their shape is different, however. In Figure 2, A, it is clear that the normal distribution of the lines is fairly evenly spaced across the sheet, at any given height, hence their relation is expressed by very nearly a straight line. In Figure 2, D, the spacing is even, but up and down rather than horizontally across the page. Curves as shown in Figure 2, C, however, if properly drawn, ought to have the effect of arranging the lines *harmonically* across the page, as is desired.

If these curves are not properly drawn they lead to a variety of errors. If the curve is the wrong shape (provided the end points are right) it means that in trees of medium height the diameters are incorrect. Curves which touch the basal line fortunately have one point fixed, for diameter at the top of a tree must always be zero. The curves representing points 10 and 20 feet above the ground approximate straight lines and diverge but slightly, but higher curves are difficult to draw. In the first place, the values high in the tree are erratic, for reasons that will be shown later, and the curvings in series B do not remedy matters very much. Secondly, these points are incapable of much correction by the curves, for the change of direction is rapid at that point, divergence is considerable, and the curves are widely spaced, all of which renders the proper placing and shaping of the curve very difficult.

In practice it is found that the curves are most naturally drawn to pass through the point plotted to represent diameter 10 feet from the top, while actually such points often need severe correction. An error in diameter 10 feet below the top may not exist in one diameter class alone, as such a possibility has already been ironed out in the curves of Figure 2, B. Consequently, curves of Figure 2, C, pass it on, although theoretically they ought to remove it. For instance, in Figure 2, C, let us suppose the diameters of all the trees in the 50-foot class at the 40-foot point are high because of the misplacement of the 40-foot line in the previous curving of the type shown in Figure 2, B (for the regularity of the spacing of these lines is difficult to judge on a large scale). Then in Figure 2, C, the point A will fall high at the 40-foot point. In 40-foot and 60-foot tree classes (points B and C) the values we will suppose to be correct. Nevertheless, because of the rapid curvature, lack of parallelism and width of spacing in that part of the graphs, it is hard to make a choice between the solid line or the dotted line as shown in the figure, one confirming an erroneous value and changing a correct one, the other changing the wrong value to the right one. Curves of the form shown in Figure 2, C, serve to even up the erratic values, however, especially in tall trees and in the lower part of the bole, and to make changes run smoothly from height class to height class within the same inch class.

As each inch class is adjusted separately, there is no assurance that the same relative adjustment will be made in the neighboring inch classes. Accordingly, values are read back again to make curves of the type shown in Figure 2, B, where the inch classes are again put in harmonious relations with each other, with some possible disarrangement of ideal relations existing between the different height classes. These curves are finally transformed back again to curves of the type shown in Figure 2, A, the finished taper curve.

Having briefly outlined the method and noted some of the difficulties encountered, its practical effectiveness must be considered. The first curving makes the form of the trees in each diameter and height class independently smooth, although their forms may differ. This is good. Next, the lines are spaced evenly and are made to run through the right d. b. h. through the process shown in Figure 2, B. But although spaced evenly, what is the assurance that the spaces are the right distance apart? If wrong in a single height class, curves of series 3 (fig. 2, C) will tend to iron out the trouble when the erroneous value is placed in comparison with correct values in adjacent classes. But just here is where one error comes in.

In practice, the first curves fail to converge as they are shown doing in

Figure 2, A, upon the exact height class. Seventy feet may be tall for 9-inch trees and the average height of the 9 inch–70 foot class may be only 67 feet. Likewise, 70 feet may be short for a 16-inch tree and the average height may be 72 feet. So, ultimately, in a given diameter class, short trees are forcibly stretched out to the mean of their height class and tall trees are pulled down to the mean of the class, as already brought out and shown in Figure 1 and Figure 3 A, an idealized form of Figure 2 D, which leads to a distortion of form.

In practice, in drawing the first curves of the form shown in Figure 2 A, an irregular jumble and crisscross-

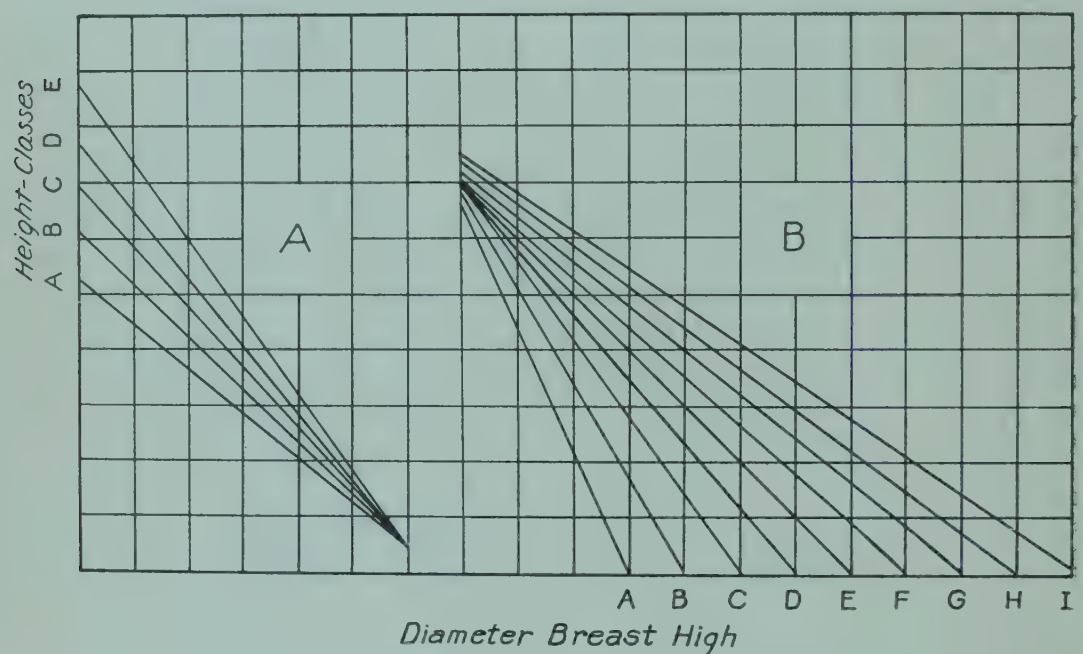

FIG. 3.—Failure of all taper curves in the same height class to converge upon the mean of the class

ing of lines in the upper part of the graph always occurs. Theoretically the result should look something like Figure 3 B, but owing to miscellaneous errors and differences in form toward the tree tips, instead of a mere failure of the lines to converge on the mean height value of the class as shown, there is a tremendous tangle of crossing lines, for there may easily be 20 inch classes in a single height class. When this is straightened out graphically and each curve forced to end at the mean value of the height class, it results in a much wider spread of values toward the top than there should be. The second curving thus straightens out the criss-crossing, but leaves the total spread of the lines about the same; the spacing is made even, but abnormally wide, so that small trees are forced to take on abnormally slight taper toward the top and big trees are made to show a great taper. The effect extends to a certain degree all the way down to breast height and tends to make the diameters of small trees (short for their height class) run small, and large trees (tall for their height class) run high. All this tends to make trees in extreme size classes show a form they do not actually possess.

In the next curving (fig. 2, C) values of trees in the same diameter class but in different height classes are combined. On every height line above the ground, values will run low in the short trees and high in tall, which merely shifts the tilt of the curves, a matter which is absolutely invisible when all are tilted equally or even harmonically. So this error passes on through to the end. Thus incorrect values caused by wrong spacing for any reason whatsoever of lines in Figure 2 A, tend to persist, as actually occurred in one instance of lodgepole pine taper curves.

The space between the lines is greater in the 70-foot class than in any other in the finished taper curve. This could be avoided to a certain degree by harmonizing the curves on a different basis to eliminate the inefficient third curve. Several alternative methods investigated by the writer, however, while more sound theoretically on account of the use of almost straight line curves throughout, proved practically as unsatisfactory in actual use and involved a great deal more labor than the system of harmonization outlined by Barrows.

The only conclusion possible from this study of Barrows's taper curve construction is that by it are readily obtained harmonized values that change

smoothly from class to class. The system, however, can not claim a great degree of accuracy. In fact, it tends to sacrifice accuracy to harmony. To work best, not only a large basis of individual measurements is needed, but they must be well distributed through many diameter and height classes so that the resulting curves may be long and their trend be clearly shown, minimizing the ever present danger of tilting the curves into incorrect positions.

The object of this criticism of the method of preparing taper curves is not to throw doubt upon the accuracy of the volume tables prepared in accordance with this system (which have proven fairly satisfactory) but to show that it is not by any means perfect and that equal accuracy may be obtained by simpler means.

FRUSTUM FORM FACTORS—BRUCE'S METHOD

One obvious way of getting around the difficulties of Barrows's method is to set a standard form of tree and compare all others with it. This is the essence of Bruce's frustum form factor idea (*4*).

The difference between the volume of a tree and the volume of a frustum of a cone having the same top diameter and the same d. b. h. ought to vary slowly and consistently with the changes of diameter, height, and form. Sudden changes are inconceivable where average trees are concerned. The frustum form factor method of volume table construction has been employed by Bruce and has proven most excellent indeed. It is easy, quick, and, as demonstrated by Bruce and others, is surprisingly accurate (*5*, *7*, *10*). It has the disadvantage, however, of what may be termed inflexibility. The final results come in one step from the basic data, and if there are any other results desired they must be worked up anew from the original measurements.

If a volume table is desired running to a 6-inch top limit, the volume of each tree to that limit must be figured and compared with the corresponding frustum. The frustum form factors must be averaged, and the computation for volume be made from them. If now a new table is desired showing volume to a 7-inch limit, the whole process must be repeated. With taper curves, the preparation of new tables is a matter of minutes. Furthermore, the taper curve system is the only one applicable to linear products, ties, props, etc.

Bruce's frustum form factor is an empirical sort of figure bearing little relation to other usually accepted tree form constants, as form factor, form quotient, form exponent. One reason is that diameter breast high outside of bark is made one of the points through which the frustum surface passes, so that the frustum form factor will vary with bark thickness, if all other factors as height, diameter, and form remain constant. Thus it is not a measure of form in the sense that some other factors are.

The frustum form factor values also will vary with the top cutting limit used, because the lower part of a tree has more conical taper and the frustum of a tree cut to a 10-inch limit will fit much more closely to the frustum of a cone than when the top limit is 6 inches, well in the top of the tree. It is possible for two tree frustums of different form and equal top and basal diameters to have the same volumes, and hence the same frustum form factors. This factor is thus an expression of volume relations rather than form relations. It is a very useful empirical figure, and the basic idea is sound. Nevertheless it fails to fill the place occupied by the taper curves. It is still worth while to discover, if possible, improved methods of curve construction.

MATHEMATICAL EXPRESSION OF TREE FORM

A simple mathematical expression of form, and a generalized equation of tree curves has been sought by European foresters for many years, but none has proven entirely satisfactory (*8*). There have been no attempts of this kind in America except the recent modification of the Höjer formula, worked out by Behre (*3*) for western yellow pine in Idaho, which is of too recent introduction to have proved its general usefulness as yet. The discovery of a simple general curve equation of this kind would go far in solving tree mensuration problems. In the search for such an equation, however, certain facts have been discovered which pave the way for a much simpler method of expressing tree form through taper curves.

PROPOSED SUBORDINATE FORM QUOTIENT METHOD

Tor Jonson, according to Claughton-Wallin, has proved that the taper of the trees of the same form class (form quotient) is independent of height with certain north European conifers. (The

fact that the taper is the same for trees of all diameters within the same height and form class had already been proved by Maass, Schiffel, and others.) Whether Jonson's dictum is strictly true or not is of little moment. It is sufficient to say that by application of this hypothesis he has developed a wonderfully accurate system of timber estimating in Sweden. If accurate enough for European conditions, it should be ample for our needs.

Accepting Jonson's statement, therefore, that taper of trees of the same form quotient is the same from the top to bottom, we have only to gather all trees of the same form quotient together and determine the diameter at regular intervals up the stem in each class. base of the tree is considered to be at breast height, and the middle diameter is at a point halfway between breast height and the tip of the tree.

Given the usual series of taper measurements, as collected in the field, the method of curve construction is not difficult. The different steps are illustrated by actual cases taken from the preparation of curves for Douglas fir in southwestern Idaho, which have been built by this method.

These taper curves were built from a slender basis of trees as an experiment to see how the new method would handle a difficult case. The basis was 1,123 trees scattered from the 12 inch–60 foot class to the 48 inch–150 foot class, a total of 136

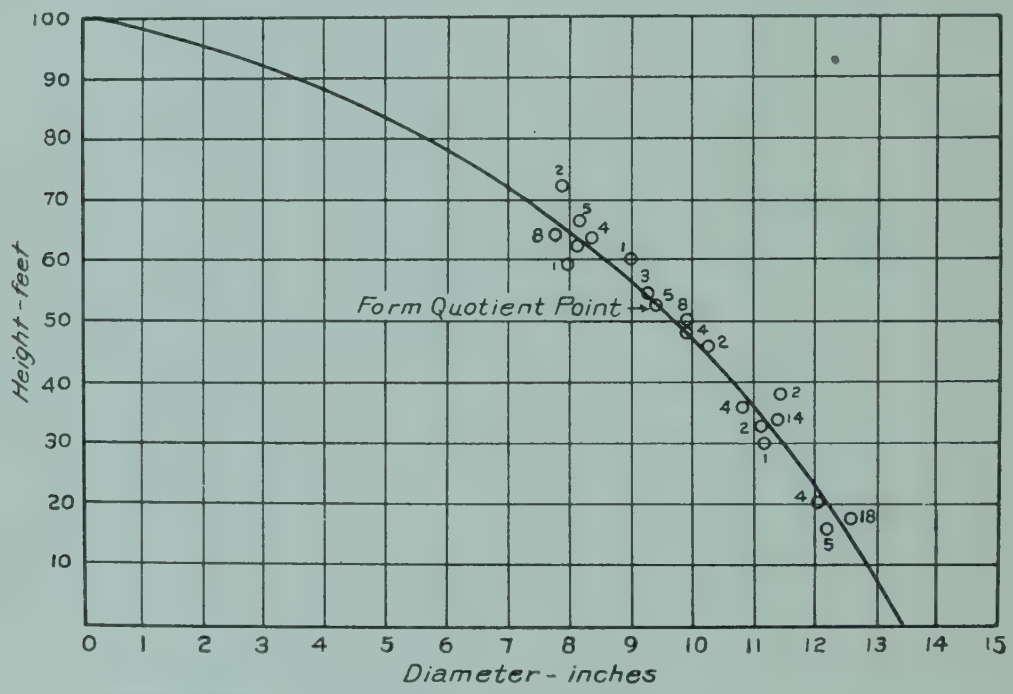

FIG. 4.—Crude taper curve for 16 inch–100 foot class Douglas fir. Numbers at points indicate number of measurements averaged

Since trees of various heights are thrown together, the points of measurement must be at equal fractions of the tree height; and since various diameters are thrown together, the diameter at any point must be expressed in terms of diameter at the base of the tree (or some other fixed point). These figures, expressing diameter at various fractions of total tree height in terms of basal diameter, are very similar to the form quotient, which is this figure in the special case when the upper diameter is taken at half the height. Diameters at other points expressed in terms of basal diameter may with propriety be designated as subordinate form quotients. It must be remembered that here we are dealing all along with *absolute* form quotients in which the different individual diameter-height classes being involved, or an average of only about 8 trees to a class. The largest single-size group—the 16 inch–90 foot class—contained only 35 trees; and 35 classes were represented only by single trees. It is obvious that a difficult test was imposed. It must be borne in mind also that the exact figures and form relationships found are not to be considered as fundamental and must not be used as the foundation of any generalized concepts of tree form—even of Douglas fir, for there are, as a matter of fact, a number of fundamental details that appear unsound. This work is to be considered only for what it is—an attempt to use a new method in building up a practical set of taper curves.

The sheets carrying the original data gathered in the field are sorted in the usual way into diameter and height classes. The diameters are then averaged and taper curves are drawn for each height class, based on diameters inside of the bark (fig. 4). Inside diameters are almost never taken at breast height. Accordingly, the curve is simply brought down from the first measurement to the base line of the graph by eye. The stump diameters should not be plotted, and no stump flare or basal swelling be allowed for, as it will tend to cause later difficulties. It is indeed much better to leave out the actual diameter inside of the bark at breast height, even if the figure is available, as on large trees it may be affected by stump swelling and introduce error into the curves at this time.

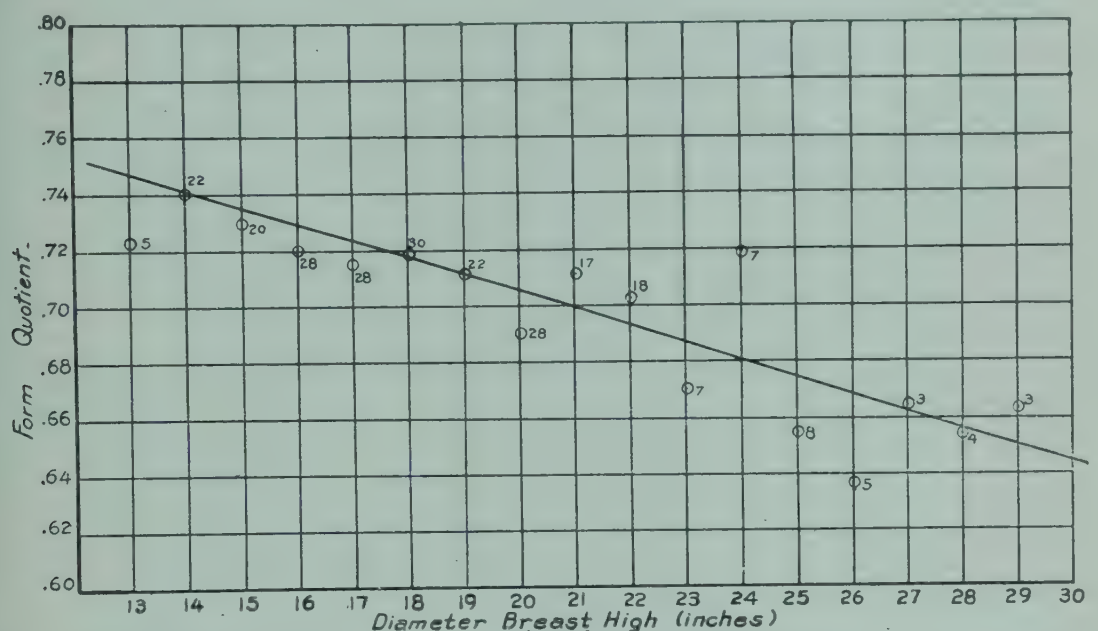

FIG. 5.—Hundred-foot class Douglas fir, showing method of smoothing irregular form-quotient values Numbers at points indicate number of trees as basis

Later it will be easy to take care of any peculiarities in d. b. h. measurements due to any cause. It is not at all necessary that the average diameter in any group should fall at the even-inch class or that the average height should fall upon the mean height of the class. After the rough diameter-inside-bark taper curve is drawn, the diameter at a point one-half way between breast height and the tip of the tree is found and then divided by d. b. h. inside bark as read from the graph. This gives the assumed absolute form quotient. After form quotients for all the size classes have been computed the values are evened off by curves.

The propriety of assigning to each size class its appropriate form quotient which shall be in harmony with that of neighboring size classes, is open to a certain amount of scientific criticism. In Europe, form is generally held to vary with the density of the stand and to vary almost as widely in a single size class as in a whole stand. It therefore becomes most proper to assign a single form quotient to the whole stand on the basis of density. Possibly we would do well enough by simply throwing all size classes together and taking a single average form quotient. In the work done by the writer, however, there is a fairly regular trend of form-quotient values according to size, the quotients falling as diameter increases (in Douglas fir) and increasing with height. Possibly this is due in part to a rough correlation of size with density in many of our unmanaged forests; but, whatever the cause may be, the result is quite apparent, and consequently the form quotients of the various size classes have been harmonized by curves.

The values for each height class may first be conveniently smoothed separately, as the form quotient tends to change very slowly within the same height class with the species studied by the author. (See fig. 5 for the 100-foot class, South Idaho Douglas fir.) There are also many diameters in each height class, which gives much longer curves than would be obtained if an attempt were made to draw curves for each diameter class separately. All of these curves should then be plotted upon a single sheet so that the relations between height classes may be judged, and any necessary adjustments made, for it is obvious that values should not run

irregularly from height class to height class. In all the work done by the author little adjustment of this kind has been necessary.

From these final curves the true absolute form quotient for each size class may be read. The next step is to select a number of the original taper curves which have a good basis in number of trees and yet show a wide variation in form quotients. After selecting 20 or 30 of these, the distance between breast height and the tip of the tree should be divided into 10 equal parts (fig. 6). The diameter of each one of these points up the tree is then read off and put down on paper. After this is done, each of these diameters is divided by the d. b. h. inside bark of the tree from which they were taken, this being done very rapidly and with ample accuracy by slide rule. In this way a series of subordinate form quotients are obtained, showing the diameter at 10 points equidistant up the tree in percentage of diameter breast high.

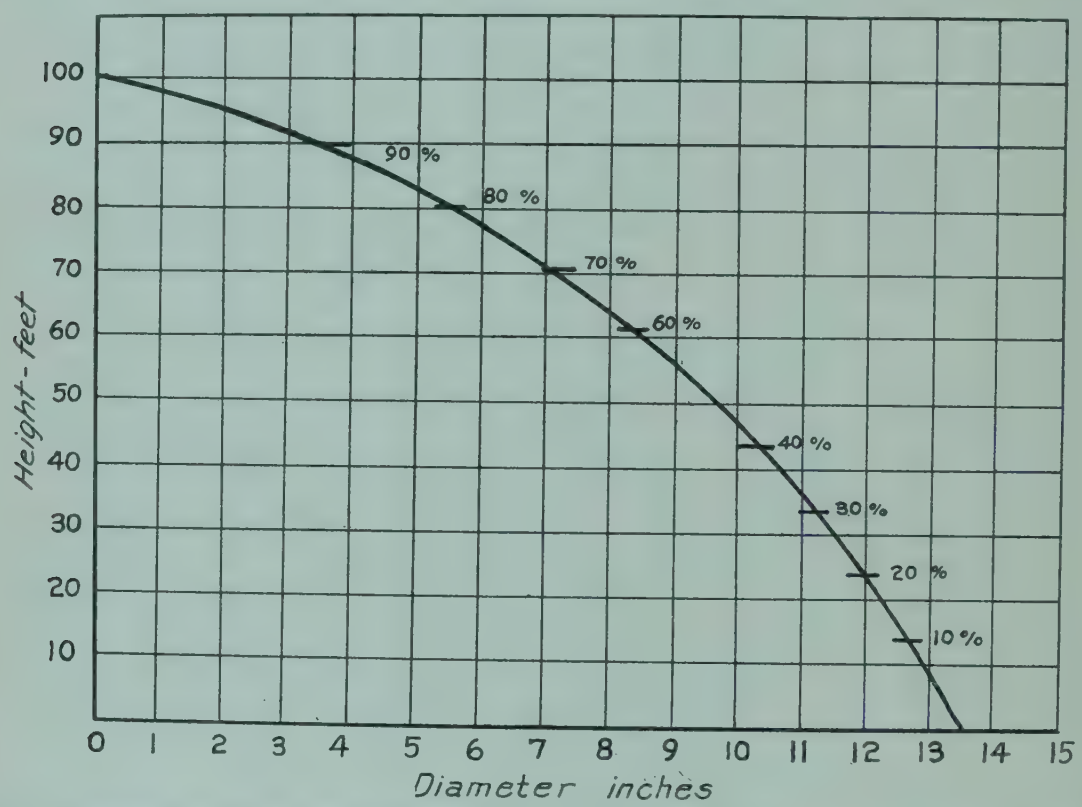

FIG. 6.—Diameter inside bark curve subdivided to determine subordinate form quotients for 16 inch–100 foot class Douglas fir

In order to even off these values a series of curves should be made, with the form quotient as the abscissa and the subordinate percentages or form quotients the ordinate. All the values which have been computed should be plotted on a single graph according to the appropriate form quotient of the tree, as shown in Figure 7. In the example of Douglas fir, here used, they fall into very good lines and require little study in order to draw the proper curves. The curve at 0.5 of the way up the tree must be a straight line because that is the form quotient line. The lines representing the diameter percentage at 40, 30, 20, and 10 per cent of the height of the tree also apparently fall in straight lines, which when extended meet at ordinate value 1.0 and abscissa value 1.0. At 60, 70, 80, and 90 per cent of the tree height they are flat smooth curves.

After drawing these curves it is possible to read off the proper subordinate form quotients for any diameter and height class after reading the proper form quotient from the curves already prepared (as in fig. 5 for the 100-foot class). It is a simple thing then to prepare a taper table if the proper base diameter is known for each d. b. h. class of trees. This can easily be ascertained by plotting the d. b. h. inside the bark from the curves first drawn against the d. b. h. outside the bark for each diameter class (fig. 8). Variations can easily be smoothed out by simple curving.

Now, having the correct diameter inside the bark, the correct form quotient, and the correct subordinate form quotients all the way up the tree, it is a simple matter to draw smooth and correct taper curves by applying the

appropriate percentage to the proper diameter inside the bark (fig. 9). To anybody familiar with curves of similar form made by the conventional system, a striking difference will be noted in the upper part of the tree, where all the curves converge into one. A wide spread is the rule in the curves made by the usual system. In effect, this harmonize the whole curves themselves by the graphic methods employed by Barrows. The results are, furthermore, very much more dependable. This method also obviates any necessity of working out mathematically the equation of these curves.

It is interesting to compare the taper found by the method here used for

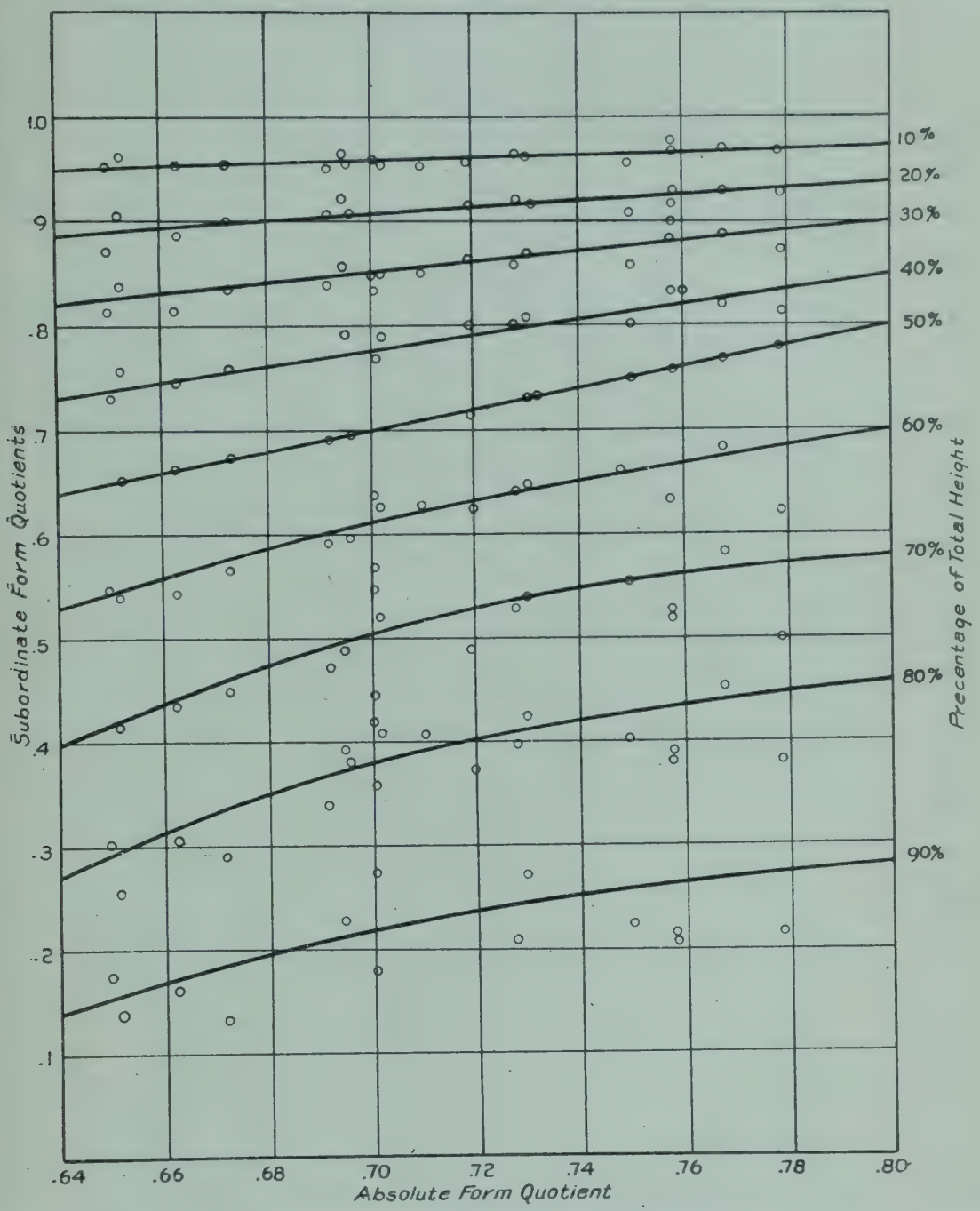

FIG. 7.—Subordinate form quotients at ten equal intervals from the base of the tree to the top for Douglas fir trees having form quotients from 0.64 to 0.80

method gives the form or taper curve of all trees having the same form quotient, and each size class of trees is assigned its tree form simply by working the form quotient into a regular, orderly sequence. It is obviously much easier to bring these simple figures into order than to arrange, rearrange, and

Douglas fir and the taper found by Behre for western yellow pine, using his modification of Höjer's equation. The difference is very slight. Taking Behre's form class 70 and comparing it with the values for form quotient .700 in Table I, which is based on the most complete data, the following results are obtained.

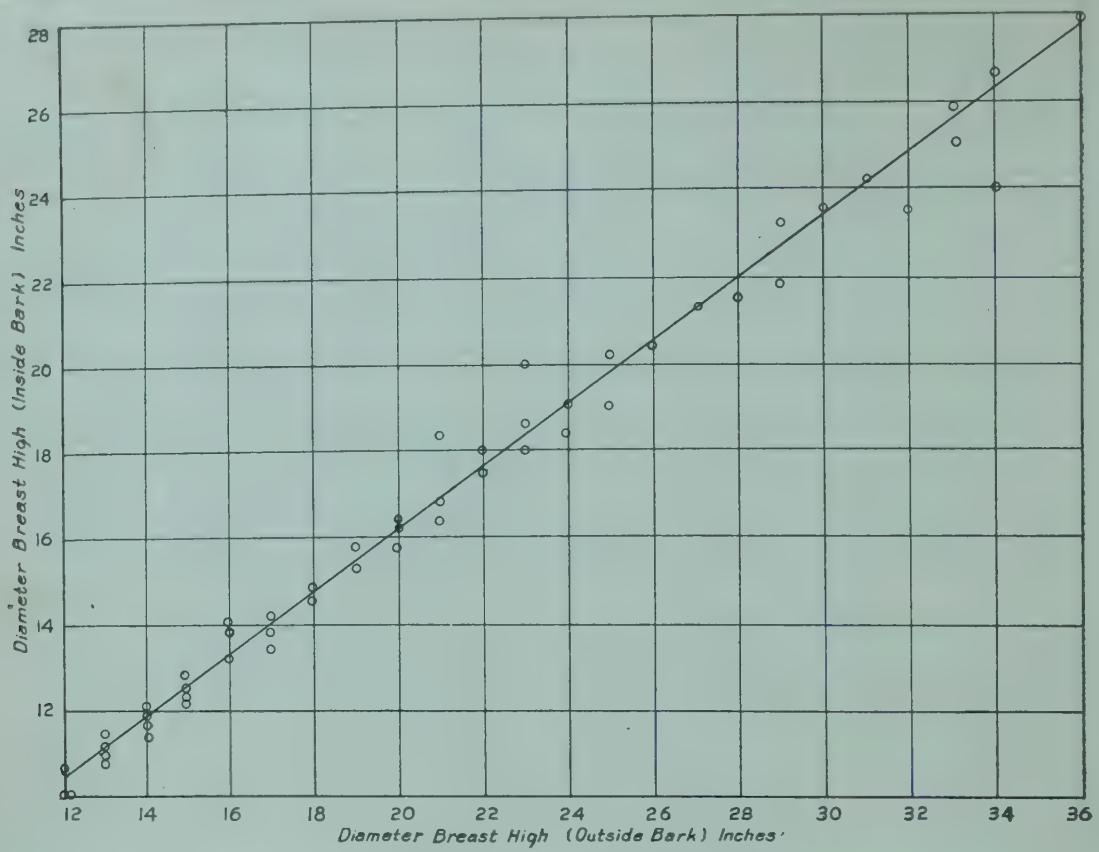

FIG. 8.—Relation of d. b. h. outside of bark to d. b. h. inside of bark

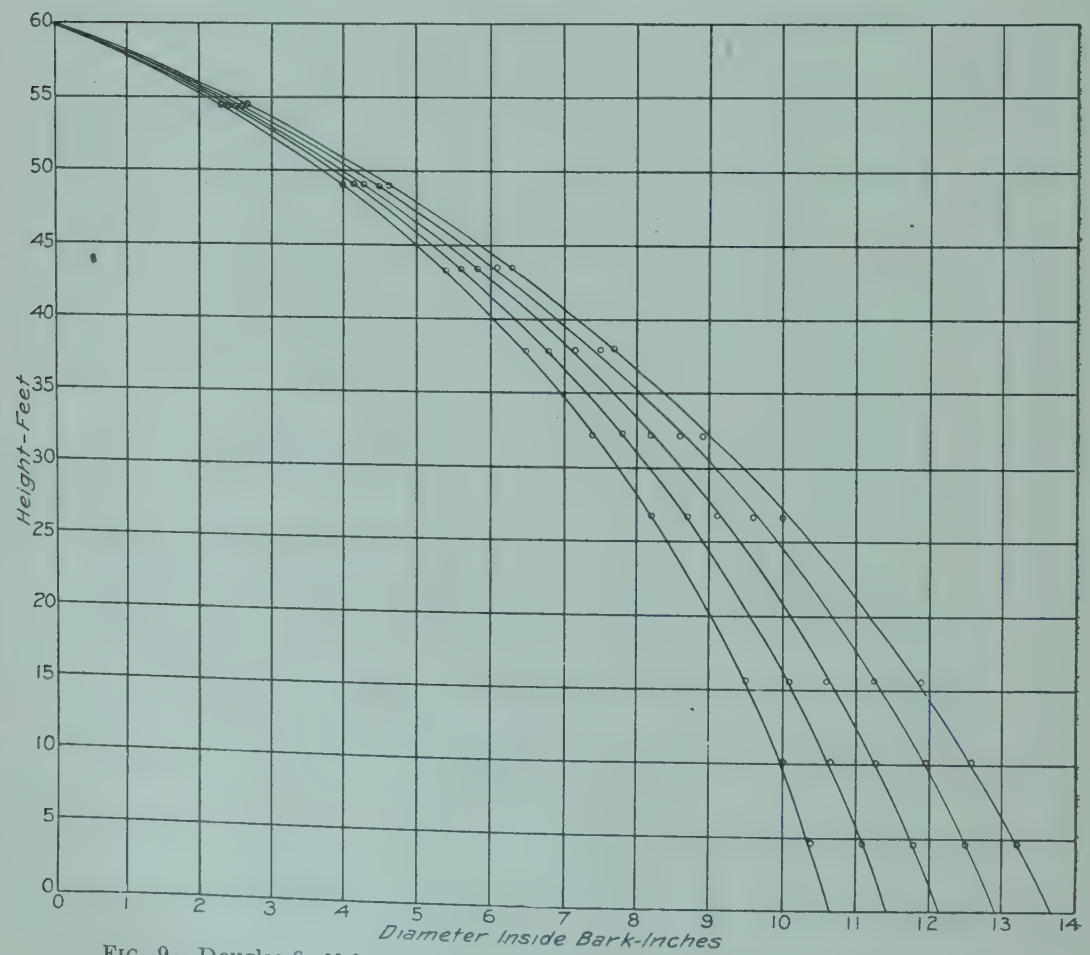

FIG. 9.—Douglas fir 60-foot class taper curves (12 to 16 inches d. b. h. inside bark)

TABLE I.—*Comparison of taper for Behre's western yellow pine form class with the taper for Douglas fir, form quotient .700*

Percentage of length from tip to breast height	Percentage of d. b. h. (inside bark)		Percentage of length from tip to breast height	Percentage of d. b. h. (inside bark)	
	Western yellow pine [a]	Douglas fir [b]		Western yellow pine [a]	Douglas fir [b]
90	95.5	96.0	40	60.9	61.2
80	90.3	90.6	30	50.1	50.5
70	84.5	85.0	20	36.9	37.8
60	77.7	77.5	10	20.6	21.5
50	70.0	70.0			

[a] Behre. [b] Baker.

TAPER CURVES BASED ON THE PARABOLOID FORMULA

The system of curve construction just described was evolved because a still simpler system failed to work on Douglas fir, although it gave excellent results with both lodgepole pine and aspen. This involves the assumption that the taper curve follows the generalized formula for a paraboloid: $Y=px^n$, or as it is more often written in forestry literature, $Y^2=px^r$. In this equation Y=diameter, x=distance from the top of the tree, and p and r are constants. This formula gives a series of parabolic curves varying in shape with the value of r, and in steepness with the values of p.

The first question that naturally arises is, how much risk of introducing error we take by the use of this hypothesis. Judging from Graves's "Mensuration," (*9*) it is used widely in all Europe, and exists in an implied or approximate form in many formulas for determining the volume of trees. Table II is presented to show the comparison of the theoretical form with the actual average form in the case of the 60-foot height class in lodgepole pine. The curve $Y^2=px^r$ was based upon d. b. h. and d. h/2 in this work. Column A gives the actual average values (unharmonized by curves) in each class, column B the theoretical values. It is obvious that neither is an absolute value; there is a probable error in each. This has been figured for the actual values and it runs very close to ± 0.1 inch, except in the 14-inch class, where it is nearer ± 0.2 inch. The probable errors of the computed values in columns B are of approximately equal magnitude.

It is very evident that the error in assuming the trees to be paraboloids lies well within the probable error of ± 0.1. Hence it is obvious that the form of lodgepole pine is virtually a paraboloid, at least in the 60-foot height class. Tests similar to that shown in the above table were made in a variety of diameter and height classes where a large number of tree measurements were available and the results were equally conclusive in every case. Accordingly, it was assumed that the

TABLE II.—*Comparison of theoretical form with the actual average form for 60-foot height class lodgepole pine*

D.b.h. (ins.)	Diameters at specified heights above stump (inches)											
	8		16		24		32		40		48	
	A	B	A	B	A	B	A	B	A	B	A	B
10	9.6	9.5	8.9	8.8	8.2	8.0	7.2	7.0	5.9	5.9	4.3	4.5
11	10.5	10.5	9.6	9.7	8.9	8.8	7.7	7.8	6.5	6.5	4.4	5.0
12	11.4	11.4	10.5	10.4	9.3	9.3	8.1	8.1	6.6	6.7	5.1	5.0
13	12.2	12.3	11.2	11.1	9.9	9.9	8.5	8.5	6.9	6.9	4.6	5.0
14	13.0	13.2	12.2	11.7	10.4	10.3	8.8	8.6	6.9	6.8	4.8	4.8

A=Actual average values, unharmonized by curves. B=Theoretical values.

same fact applied to all classes, and that any failures to agree were due to insufficient numbers of trees as a basis of computation. Curves were constructed upon the hypothesis. It may be noted in passing that these curves conform very closely to lodgepole pine taper curves already published (*11*).

In order to take advantage of this short-cut method it is only necessary to discover whether the taper curves in the size classes having the best basis in trees conform to the theoretical paraboloid curve. If they do so conform, the short method will suffice. If they do not conform, the method described before can be used. The methods of compilation start the same in either case. The original sheets are thrown into diameter and height classes as usual, the measurements inside bark are averaged, and separate rough taper curves are constructed for each diameter and height class. The form quotients are then computed, and the values smoothed by curves, as in Figure 6. Then, choosing several size classes where the basis in trees is heavy, the general correspondence between the theoretical curve and the actual curve should be noted. If it is close, the general similarity of all size classes to the paraboloid can be assumed. Then if FQ=form quotient, $r=\frac{-\log FQ}{0.15}$, r being the exponent in the parabolic formula $Y^2=px^r$.

The value of r always falls near 1. Then if x equals the distance from breast height to the top of the tree and Y = diameter breast high inside bark, $Y^2 = px^r$, from which p can be ascertained. Then by use of this same equation, by introducing different values for x, the diameter at various heights can be readily ascertained.

The process sounds complicated, but can be done rapidly when once mastered, especially with the aid of an alignment chart. Such a chart is illustrated in Figure 10 (*6*). It may be mounted on a drawing board and arranged with a strip of celluloid bearing a straight line pivoted with a thumb tack moving on the FQ–R line, and a thread fastened to a pin moving on the P line (fig. 10).

To solve the equation by this chart, take any diameter and height class—say the 60-foot height class—look up the form quotient for that class and pivot a line on the celluloid strip at the appropriate value. Make this line pass through the height of the tree minus 4½ feet (the distance from the tip of the tree to the "base" at breast height) on the H line. Then take the pin and thread and stick the pin in the value of diameter breast high inside bark for the particular class you are dealing with. Make the thread intersect the line on the celluloid strip on the unlettered and ungraduated line and stretch on to intersect a certain value of p, which should be marked. Then reverse the process. Stick the pin at the marked value of p. To find the diameter of the given tree 10 feet from the tip, pivot the celluloid line till it cuts 10 on the line H. Then stretch the thread from p across the intersection of the celluloid line and the plain line, until it intersects a value on line D, which is the diameter 10 feet from the tip of the tree. The method is rapid.

The advantage of this method, as well as the one first outlined, over the older method lies in their ability to iron out errors (especially those due to failure of the data in any diameter and height class), and to average up to the middle of the class, while the results are expressed in the same useful taper curves. Their advantage over the system of frustum form factors lies in the fact that they have the same ability to get accuracy from scant data and are only slightly more laborious, while the results are expressed in taper curves instead of board feet. The wide usefulness of taper curves in all kinds of volume computation, yield, etc., is too well known to need enlarging upon. The last method is also useful, for if trees are proven paraboloids, a very simple relation exists between form quotients and the regular conventional form factors, by the use of which total cubic contents can be very easily computed.

CONCLUSION

The whole subject of tree form needs deeper study so that the fundamental laws may be learned, which will lead to further simplification of methods in volume table construction. This study represents only the development of a simple but nevertheless empirical method based upon what is known at present of tree form, i.e., that trees of a given species having the same form quotients have the same form from top to bottom (excluding basal flare), and the very safe hypothesis that form (as expressed by the form quotient) varies regularly with changes in diameter and height.

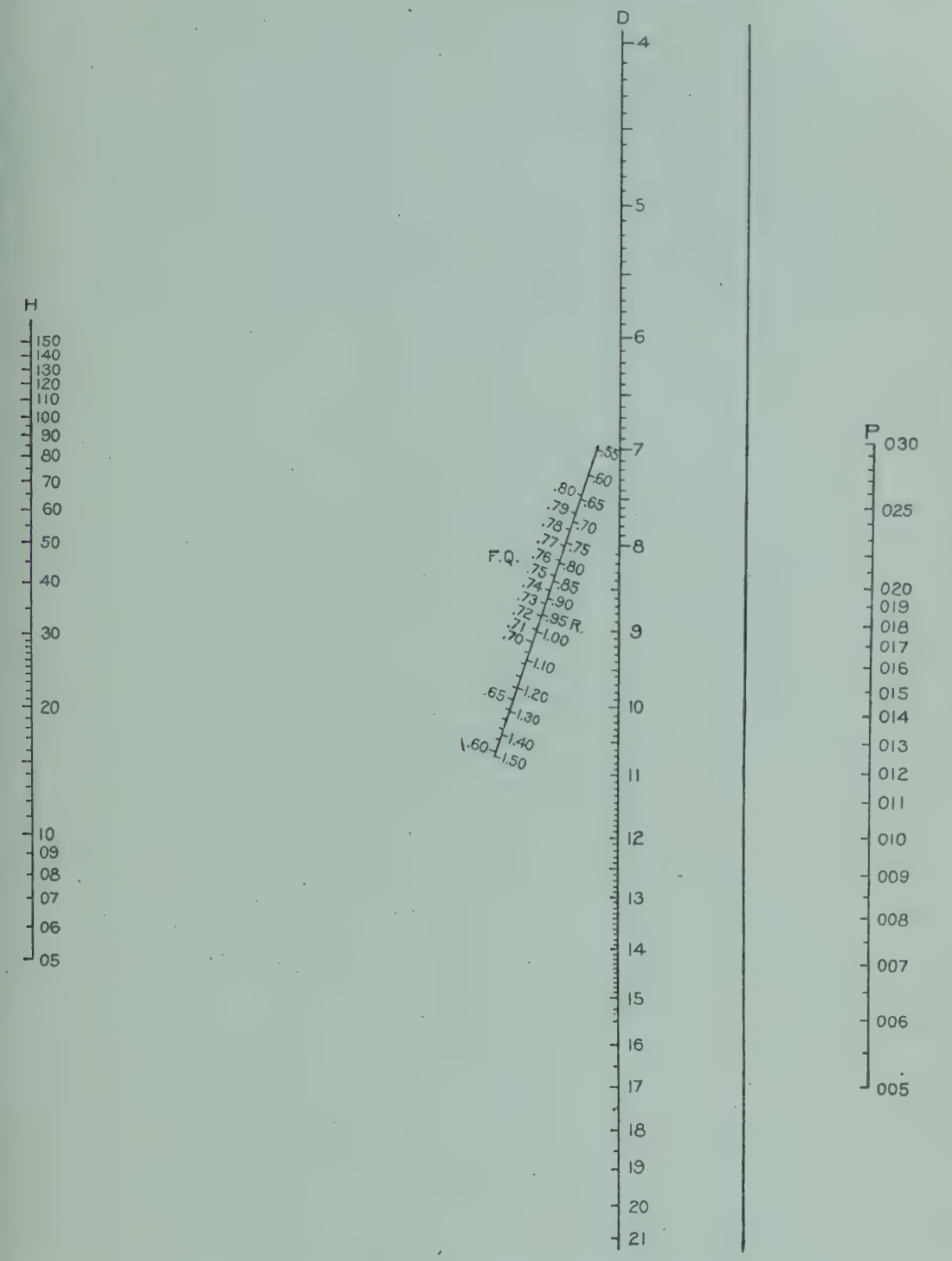

FIG. 10.—Alignment chart for solution of equation of a parabola

$D^2 = P.H^r$

D = Diameter of point H feet from top of tree.

H = Distance from top of tree to point where D is taken.

P = Parometer. Constant in trees of same size and form.

R = Exponent. Constant in trees of same form.

FQ = Form quotient D of point halfway between $B.\ H.$ and top ÷ D B H. Constant in trees of same form.

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PSILOCYBE AS A FERMENTING AGENT IN ORGANIC DÉBRIS [1]

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INTRODUCTION

As it comes from the sugar mill, the ground cane, or bagasse, consists of fibrous tissue and crushed parenchyma, enmeshing some of the protein, gums, and coloring substances and a trace of the sugar of the cane plant. The mass averages about 50 per cent moisture, but this moisture is principally derived from water sprayed on during the grinding process, instead of natural sap.

If not burned in the furnaces, enormous masses of bagasse accumulate at the factory. During the winter of 1924, in connection with a study of the causes of spontaneous combustion, in which The Celotex Co. coöperated, we were able to examine a large quantity of this material. Masses of bagasse at that company's mill at Marrero and at several of their baling stations at sugar mills were inspected, after they had stood in piles for from two weeks to about two months. These masses illustrate clearly the principle that the composition of any particular product determines in advance the types of fermentation which will take place in it.

Bagasse heats quickly when piled according to the methods observed. The exact agents responsible for the heating process were not determined. When these heated piles were examined, certain characteristic organisms were found. In the loose piles, mucors and Trichoderma were abundant, as examined with the hand lens. When holes were dug down from the top of the pile, fruiting mucors were observable as deep as 2 to 3 feet. The temperature of the pile rose gradually, however, until it reached 62° C. between 3 and 4 feet below the surface. Bacterial examination of material taken at this level showed typical rod forms still viable, but not in great numbers. Uneven heating processes may possibly account for the survival of occasional molds which appeared in cultures from the hot materials. If any vegetative activity continued at that temperature, it was restricted to very few and very special species.

OBSERVATIONS ON BALED LOTS

In baled lots produced for a special purpose, somewhat different observations were made. Temperatures up to 60° and 62° C. were found in the center of bales deep within the larger piles, even after standing for as long as six weeks. No higher temperatures were observed, although the possibility of higher temperatures was not excluded. These piles were fairly well ventilated.

On the surface of the bales the first organism to develop was *Monilia sitophila*, which spread over the piles with great rapidity and draped them with festoons of orange fruiting masses. Even after three to four weeks, handfuls of mycelium and spores could be gathered in many places.

Bales protected from rain appeared to be so well pasteurized by the temperature attained during the heating process, which was general and so completely dried out that further activity of microorganisms was prevented. Where exposed to rain, the looser parts of the bales showed abundant fruit of mucors and Trichoderma, mostly Trichoderma, which penetrated several inches into the ends of the bale. Such molds as the Penicillia and Aspergilli were inconspicuous when apparent at all, although their presence was demonstrated by culture.

The central areas of the bales were tightly compressed and apparently furnished conditions unfavorable for the growth of the common molds. Whenever the bales had become wet and cool these central areas were penetrated by mycelia, which, as could be seen with the naked eye, followed the cleavage lines entirely through the bales as they were split

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open. The whole mass penetrated by these mycelia assumed a characteristic yellow color.

The tops of piles about 3 to 4 weeks old often showed an abundant crop of agarics, mostly a species of Psilocybe, but including occasionally Hypholomas and Coprini. Myxomycetes were seen fruiting on the sides and even on the tops of the piles, without giving evidence or suggestion of extensive participation in the fermentation observed. Cultures of the plasmodia of one very common form upon ground bagasse produced no changes comparable to those observed in the piles. Nematodes also were abundant, but their effect upon the mass was not determined.

The two conspicuous features of the fermentation were the heating process and the yellow fermentation.

Further study by the writers has been limited to the cause of the yellowing process. The preliminary survey had suggested that the mycelium belonged to some hymenomycete. Strands and streamers of hyphae were traceable up through the bales of bagasse, almost to the base of the agarics seen upon the surface.

Cultures from samples taken from many places in these piles yielded *Monilia sitophila*, Trichoderma, mucors, *Aspergillus fumigatus*, *A. terreus*, *A. niger*, *A. flavus*, Fusarium, and an occasional Penicillium, with nematodes as a common contaminant. White mycelia, producing coiled masses of microspores, were occasionally obtained, but they were not recognized at this stage of the study. Such a variety of species in culture was clearly foreshadowed by the abundance of microorganisms on the surface of the mass. The air was so full of spores that everything handled was excessively contaminated.

Tubes and flasks of the original material were prepared with the addition of various quantities of water. In certain of these tubes of bagasse a few fruits of the mushroom type were obtained. Following the technique devised by Duggar for *Agaricus campestris*, the developing caps were dissected, and the pieces were distributed in tubes of Czapek solution agar, wort agar, bagasse, and various stock media used for bacteriological work.

From tubes of wort and Czapek agar, pure cultures were obtained, from which caps identifiable as Psilocybe were eventually grown. The mycelium of this agaric proved to be white and to produce microspores in enormous numbers in masses produced by the coiling of conidia-producing branches from hyphae near the surface of the substratum. In this way whole areas became powdery with microspores in culture and reproduced extensive areas seen in the piles. Transfers from these pure cultures back to tubes of ground bagasse developed the yellow color of the original fermentation.

Cultures of this organism have fruited freely in the laboratory during February, March, April, and May at 18° to 30° C. Mycelium developed rather slowly at 37° C. without fruiting. The structure of the fruit bodies differed markedly with the nature and density of the substratum. Loose masses of bagassè in cotton-stoppered wide-mouth flasks were well overgrown with hyphae and powdery with masses of microspores, but they produced no caps. Wort agar slants were first covered with white mycelium with abundant microspores; then they occasionally produced clusters of very small caps.

On such media little buds, densely crowded along the stipes of the larger caps, appeared occasionally and produced pilei 3 to 4 mm. in diameter. Upon masses of bagasse and agar so wet as to be jellylike the mycelium remained on the surface and the pilei produced were borne upon delicate stipes almost free from surface fibrils. These pilei were thin, bell-shaped, and Psathyrella-like in appearance and texture, although the lamellae were adnexed or decurrent by a tooth as in typical forms. All sorts of monstrous forms occur upon these wet media. Globose or subglobose masses which never opened up as pilei were observed in wet agar cultures. When broken open, these often showed fully formed gills and ripe spores. On dry, closely-packed fiber, the fruits tend to be solitary and resemble fairly closely those gathered in the field.

Search of the literature of Psilocybe and related genera for the identification of this form involves many difficulties. In general, the material available is unsatisfactory because of the changes due to drying in this group and the lack of notes upon large numbers of fresh specimens. In the herbarium of the New York Botanical Garden, however, a packet of specimens collected by Earle at Auburn, Ala., was found accompanied by long-hand descriptions which tally with the writers' observations. Comparison of the spores under the microscope pointed toward the identity of the writers' material with Earle's, which had been designated a new

cies by him, but had been relabeled *locybe atomatoides* Peck by Murrill. at name may, therefore, be tentaly attached to the material here ussed. Since the conditions of wth make great differences in such a n as this, the following description n the material as collected and rown in pure culture by the writers ppended:

LOCYBE ATOMATOIDES PECK (PROVISIONAL IDENTIFICATION)

on piles of bagasse and in culture, solitary to ly caespitose, especially in rich culture media. s from very small to 3 cm. or even 5 cm. in eter, convex, slightly umbonate, to subanulate at first, becoming nearly plane or ly depressed in center in age, in watery media than 10 mm. in diameter and persistently anulate, almost Psathyrella-like; when wet ophanous, fawn color to avellaneous, becoming er shades in drier areas and in age, with surface th in center, becoming striately rugose at in, and becoming concentrically rugose on g; flesh thin white, not over 1 mm. thick. broad, rounded behind, adnexed to decurrent tooth and with decurrent lines visible as far as in larger specimens, with margin white, withcystidia, becoming purple brown with the ing of the spores. Stipe varying in length diameter with the size of the fruit, up to 3 to n. in diameter, in the largest forms hollow or ed, in smaller forms solid or nearly so, from short up to 5 to 6 cm. long in drier substrata, lose scaly at base and up to the indistinct and evanescent ring formed by remains of the ersal veil, almost smooth above the ring, in r substrata the whole stipe indistinctly and escently fibrillose, often almost smooth. es 9 to 12 by 5.5 to 8 μ, commonly about 10 by urple brown (purple visible under microscope), enly elliptical or somewhat rhomboidal, late at one end, with a pore or thin place in vall at the other. Microspores borne upon hes from the mycelium which become coiled s of oidia, 15 to 30 by about 25 μ.

s observed in Louisiana, thousands ns of bagasse piled at widely sepad mills were involved in this fertation. During the first few weeks nese piles the mycelium of Psilo- penetrates enormous masses of us material and gives the yellow to all areas attacked. The prenary heating is clearly high enough estroy infection in the center of the , even if initially present. The sion by mycelium must, therefore, n at the surface and progress in-. An organism abundant enough sure such generalized presence over rge region and active enough to uce the wholesale results observed be considered as one of the greatgents of fermentation ever disred. Further, this discovery di- attention to the possibilities of nycelium of the agariceae as fering agents. In this particular there was great rapidity in its d to involve large masses of ma-. The mycelium could be fol- through masses 2 feet or more in thickness and often it apparently continued downward through successive bales. The whole fermentation developed very rapidly. These activities were largely conducted in the denser areas of masses in which the growth of the common types of mold were reduced or excluded, apparently by lack of oxygen.

All of these observations harmonize with the known facts in the growth of wood-destroying types and the physical conditions which seem to surround the vegetative activity of the mushrooms in general. The significance of the series of observations lies in the clear demonstration of the enormous possibilities of vegetative activity by species of Psilocybe or of such better known groups as Hypholoma, Agaricus, Coprinus, or Cortinarius.

It is well known that agarics are widely present in the soil, as proved by fruiting caps regularly or occasionally found. One of the authors has collected *Agaricus rodmani* from the same spot along the sidewalk in Washington each spring and fall for nearly ten years, under circumstances indicating dependence for ability to fruit upon a particular balance of temperature and moisture. *Entoloma grayanum* Peck (determined by Peck) was collected from one spot in Connecticut each year for nine years. In the same way, numerous clusters of many species have been followed over periods of several years each. Everyone has observed the continuance of fairy-ring mushrooms in particular areas. All of these observations remind us of the existence of mycelia ramifying widely through the soil. Such mycelia are assumed to continue their vegetative activity over long periods and to penetrate into the deeper layers of the soil. The absence of the mushroom type of fruit is not evidence that the organisms are not present. In this case microspore production was abundant under conditions preventing mushroom formation.

Various species of Psilocybe are described, as from lawns, from clumps of mosses, and very generally from manure piles or richly fertilized ground. *Psilocybe atomatoides*, as represented in the herbarium of the New York Botanical Garden, has been found abundantly in connection with manure piles; hence it may be expected anywhere in richly fertilized soil. If the identification tentatively made here is correct, this species is capable of tremendous activity as a disintegrating agent acting upon vegetable remains in the soil.

CONCLUSIONS

Study of the fermentations occurring in large masses of organic débris (bagasse) led to the isolation of a species of Psilocybe capable of producing fermentation in this product. Mycelia were found to penetrate compact areas in bagasse where common molds were unable to develop. This development was extremely rapid and was accompanied by easily determinable visu changes in the fibrous mass.

The great activity shown by th Psilocybe in pure culture and in th field, considered together with th abundance of such forms in connectio with decaying plant remains, suggest the need of intensive study of th habits and possibilities of the mush rooms as agents of decompositio especially in the soil.

ELATIVE SUSCEPTIBILITY OF CITRUS VARIETIES TO ATTACK BY GLOEOSPORIUM LIMETTICOLUM (CLAUSEN) [1]

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INTRODUCTION

The lime wither-tip disease is caused an actively parasitic fungus (*Gloeo-rium limetticolum* Clausen) which tacks in their young or tender stages e leaves, stems, flowers, and fruits of e variety of lime known variously West Indian, Mexican, Common orida, or Key lime. This disease d the fungus causing it must be finitely distinguished from the longer own "citrus wither tip" and the ngus to which it has been generally tributed, *Colletotrichum gloeospori-des* (Penz.). The latter seems to be most cases a secondary invader ther than an active pathogen. lausen [2] has called attention to mor-nological, cultural, and pathogenic fferences in these two fungi. The riter has verified Clausen's main ndings in this regard, and concurs in lausen's view that earlier writers ncluded in somewhat confusing fashion wo very different fungi and their ffects in their discussions of wither tip f Citrus.[3] This confusion is not alto-ether surprising in view of the varia-ons which may occur in what is com-nonly regarded as *Colletotrichum gloeo-porioides*,[4] but at the same time the wo diseases have many points of ifference in their grove manifestation, nd the evidence is convincing that ne lime wither-tip disease is caused by distinct species, and not by a strain f *C. gloeosporioides* having unusual athogenic abilities. Winston,[5] in con-ection with his studies of citrus ear stain, was unable to find, among umerous strains of *C. gloeosporioides* ested, any indication of active patho-enicity on very young fruits of various itrus species.

Severe losses sustained by growers of limes in southern Florida, especially on the Keys, led to an investigation of the lime wither-tip disease and means of control. Spraying with copper-containing materials, such as Bordeaux mixture or Burgundy mixture, has not proved to be entirely successful, due mainly to the fact that infection spreads very rapidly during rainy weather, especially on very tender new shoots that are just expanding or on the blossom parts or on the fruit when quite small (less than ⅜ inch in transverse diameter), thus making it difficult or impossible to apply with safety a protective spray-coating at the time when most needed. Secondary drawbacks to spraying may arise from the close and irregular spacing of the lime trees, from the rough topography of much of the land, from the relatively low value of the crop per tree, and from the necessity for special control of scale insects after using copper-containing sprays. Bordeaux mixture plus ½ to 1 per cent oil in the form of emulsion has given more satisfactory results in lime wither-tip control than straight Bordeaux mixture.

It would be very desirable to find some variety of Citrus immune to attack by *Gloeosporium limetticolum* that might be grown as a substitute crop for the West Indian lime, with due consideration of the requirements for citric acid and essential oil production and for beverage use. Accordingly, a rather comprehensive investigation was undertaken along this line, with the hope that it might serve as a basis for further work by horticulturists, plant breeders, or plant pathologists looking toward the final solution of the lime wither-tip problem through

[1] Received for publication June 23, 1924; issued June, 1925.

[2] CLAUSEN, R. E. A NEW FUNGUS CONCERNED IN WITHER TIP OF VARIETIES OF CITRUS MEDICA. Phytopathology 2: 217-234, illus. 1912.

[3] ROLFS, P. H. WITHER-TIP AND OTHER DISEASES OF CITRUS TREES AND FRUITS CAUSED BY COLLETOTRICHUM GLOEOSPORIOIDES. U. S. Dept. Agr., Bur. Plant Indus. Bul. 52, 20 p., illus. 1904.

[4] BURGER, O. F. VARIATIONS IN COLLETOTRICHUM GLOEOSPORIOIDES. Jour. Agr. Research 20: 723-736, illus. 1921.

[5] WINSTON, J. R. TEAR-STAIN OF CITRUS FRUIT. U. S. Dept. Agr. Bul. 924, 12 p., illus. 1921.

the finding of a satisfactory substitute citrus variety, to be propagated by budding, by cuttings, or from seed as might prove most desirable.

The ideal would be to find a naturally immune or very highly resistant individual or race in present plantings of the West Indian lime, which is practically always propagated from seed, and so might offer possibilities along this line. The writer has observed certain differences in incidence of the disease that might indicate individual difference in susceptibility, but he has not yet observed a tree that seemed satisfactorily resistant under very severe infection conditions. Such may possibly exist somewhere, and its discovery would mean the quick reestablishment of the lime industry on a better basis in territory invaded by the lime wither-tip disease, where general economic conditions are favorable.

NATURAL INFECTION IN GROVES

That the disease is very closely confined to the West Indian variety of lime is shown by general field observations during many seasons in various parts of Florida, where the West Indian lime seems to be universally affected but where varieties of orange, grapefruit, lemon, tangerine, mandarin, citron, and even such lime varieties as Kusaie, Woglum's Seedless, Buena Vista, Tahiti, and Persian are not affected when growing in close proximity to diseased West Indian limes. The only other variety observed to be affected under natural conditions has been the Dominican Thornless (or Spineless) lime, which supposedly originated as a sport from the West India lime.[6]

FIELD TESTS

During three seasons, 1919, 1920, and 1922, field tests were made on the March growth of various citrus varieties, species, and related genera to determine possible susceptibility to the lime wither-tip disease. These tests were made on property where the disease existed naturally on West Indian and Thornless limes. All of the tested trees were within 200 feet of such naturally infected lime trees, and yet no natural infection has been observed on them during the tests or during two subsequent seasons. The inoculum was a spore suspension, usually from freshly sporulating lesions on lime shoots, but sometimes from artificial cultures. Inoculations were made by wrapping cotton swabs lightly around the very tender shoots (usually 1 to 4 inches long, with many leaves in very susceptible stages), and covering for two days with paraffin paper. Control inoculations were made for every series on susceptible limes to determine the potency of the inoculum, and favorableness of condition, and water-blank controls were also made on the limes to determine what part of the observed infection might have resulted from spores naturally present on the plant surfaces. The indications were that these last played a minor part during ordinary dry weather, as might be expected, since the shoots chosen had very recently begun to develop, and since the Gloeosporium spores depend on dew drip or rain for their distribution.

The results are given in Table I. The only evidence of any susceptibility is shown by the West Indian lime and by Dominican Thornless lime, which is a derivative of it. The tests included a representative lot of commercial citrus varieties, as well as a considerable range of unusual citrus varieties, species, and a few related genera. Puncturing did not increase materially the percentage of susceptibility for the West Indian and the Thornless limes, indicating that the shoots selected for the tests were young enough to have practically maximum susceptibility without artificial injury.

The field evidence is thus very strong that the lime wither-tip disease is closely restricted to the West Indian types of lime.

GREENHOUSE INOCULATIONS

Potted plants, three of each variety, were cut back severely so as to induce the sprouting of a considerable number of young shoots. When these were barely starting the plants were placed in an inoculation case where the air was kept practically saturated with moisture. Spore suspensions of *G. limetticolum* were atomized over these plants 10 times during 21 days. Then the young shoots, which had attained several inches in length, but were somewhat etiolated and quite succu-

[6] "In 1892 on Shelford [probably intended for "Shawford"] Estate, Dominica, a lime tree was noticed without the usual formidable spines. Seeds from this apparent "sport" were collected and sown. Some 75% came true and a plot of spineless limes forms an interesting feature of the Dominican Botanic Garden. The plants in this plot are now bearing heavily, and the Curator reports that 80 to 90% of the seedlings raised are coming true." Agricultural News, Vol. 1, No. 3, May 24, 1902, p. 38.

TABLE I.—*Field tests by artificial inoculation for susceptibility to Gloeosporium limetticolum*

Varieties tested	Unpunctured					Punctured				
	Number of tests	Total shoots	Per cent positive	Per cent doubtful	Per cent negative	Number of tests	Total shoots	Per cent positive	Per cent doubtful	Per cent negative
LIMES										
West Indian	8	53	89	0	11	3	20	90	0	10
Dominican Thornless	6	91	86	0	14	2	9	89	0	11
Tahiti	8	81	0	0	100	1	5	0	0	100
Rangpur	5	60	0	0	100	1	3	0	0	100
LIME HYBRIDS										
Limequat	8	87	0	0	100	0				
Faustrime	4	68	0	0	100	1	3	0	0	100
CITRON										
Citron of commerce	3	14	0	0	100	0				
LEMONS										
Kenedy	4	35	0	0	100	2	13	0	0	100
Villa Franca	6	44	0	0	100	3	16	0	0	100
Lamb	6	54	0	0	100	3	17	0	0	100
Dwarf Chinese	2	17	0	0	100	0				
Sweet lemon	2	13	0	0	100	0				
GRAPEFRUIT										
Marsh	7	62	0	0	100	3	15	0	0	100
Silver cluster	2	15	0	0	100	0				
Walters	5	50	0	0	100	3	14	0	0	100
Conner's Prolific	1	6	0	0	100	2	5	0	0	100
Pernambuco	4	34	0	0	100	0				
Leonardi	5	51	0	0	100	1	8	0	0	100
Foster	2	21	0	0	100	0				
Royal	3	34	0	0	100	0				
Pink Marsh (Thompson)	1	8	0	0	100	0				
Sour grapefruit	1	17	0	0	100	0				
ORANGES										
Valencia	7	60	0	0	100	3	20	0	0	100
Pineapple	3	27	0	0	100	0				
Parson Brown	4	41	0	0	100	1	11	0	0	100
Ruby Blood	2	15	0	0	100	0				
Maltese	4	31	0	0	100	0				
Homosassa	4	40	0	0	100	2	10	0	0	100
Lue	2	16	0	0	100	0				
Washington Navel	1	14	0	0	100	0				
Mediterranean Sweet	1	11	0	0	100	0				
Seedling orange	1	14	0	0	100	0				
MANDARIN GROUP										
Tangerine	6	60	0	0	100	0				
Mandarin	1	13	0	0	100	0				
Satsuma	4	34	0	0	100	2	10	0	0	100
King	2	16	0	0	100	2	10	0	0	100
Temple	2	40	0	0	100	0				
Cleopatra	2	18	0	0	100	0				
VARIOUS HYBRIDS										
Tangelo varieties	8	95	0	0	100	3	20	0	0	100
Tangelolo, pink	1	18	0	0	100	0				
Faustrimedin	0					1	1	0	0	100
Natsumikan	4	49	0	0	100	0				
Citrangequat	1	3	0	0	100	0				
Tangor	2	20	0	0	100	0				
Siamelo	2	43	0	0	100	0				
Washington Navel × grapefruit	2	19	0	0	100	0				
VARIOUS CITRUS										
Sour orange	3	27	0	0	100	1	8	0	0	100
Bittersweet orange	2	23	0	0	100	0				
Sweet Bittersweet orange	2	24	0	0	100	0				
Myrtle Leaf orange	2	25	0	0	100	0				
Rough Lemon	5	32	0	0	100	3	16	0	0	100
Citrus mitis	4	29	0	0	100	1	4	0	0	100
RELATED GENERA.										
Nagami kumquat	3	28	0	0	100	0				
Marumi kumquat	2	21	0	0	100	2	10	0	0	100
Chalcas exotica	1	7	0	0	100	0				
Severinia buxifolia	2	20	0	0	100	0				

lent, were punctured, dipped in a strong suspension of lime wither-tip spores, wrapped in wet cotton swabs, and replaced in the inoculation case for four days. Final observations were made eight days after removal from the inoculation case. Included in this test were West Indian lime, Kusaie lime, Sweet lime, Ponderosa lemon, seedling California lemon, Gold Medal grapefruit, Silver Cluster grapefruit, Pineapple orange, seedling round orange, Mandarin orange, Dancy tangerine, Sour orange, Cuban tangelo, and Mohawk orangelo.

The West Indian lime plants had all of the young shoots destroyed by wither tip during the first part of the experiment and no new growth was made. None of the other varieties was affected by the wither-tip disease during the progress of this very severe test.

In another somewhat similar test an inoculation chamber was used at an average temperature of 80° F. with the air practically saturated. Three plants of each variety were selected, all having very tender shoots with leaves just beginning to expand. These were punctured, and the inoculum, a heavy suspension of spores from lime lesions, was applied with cotton swabs. Further applications of spore suspensions were made with an atomizer on the second and the fifth day. On the sixth day the plants were removed from the inoculation chamber.

The West Indian lime plants developed 100 per cent infection. No infection developed on other varieties in the test, namely, Sweet lime, Rangpur lime, Kenedy lemon, Ponderosa lemon, seedling California lemon, Dancy tangerine, Mandarin orange, Pineapple orange, seedling Florida orange, Sour orange, Silver Cluster grapefruit, Gold Medal grapefruit.

Tests were undertaken to determine the susceptibility of a large number of plants grown under greenhouse conditions. Most of these were 1 to 3 years old, well branched, and furnishing flushes of growth that seemed normal. The inoculum was usually a freshly made spore suspension from an actively sporulating wither-tip lesion on the West Indian lime, but in some cases the spores were from pure cultures of *G. limetticolum*. Very young shoots were selected that had leaves young enough to develop infection if the plant was susceptible. In some tests the leaves were punctured some 50 times each. The spore suspension was applied on wet cotton, the shoot wrapped in paraffin paper, and the plant placed in a large glass-enclosed inoculation chamber over wet sand. Observations were made after 7 to 10 days. Control tests were carried on with each series to show the pathogenicity of the inoculum on West Indian lime. The results of these tests are summarized in Table II.

TABLE II.—*Greenhouse tests by artificial inoculation for susceptibility to Gloeosporium limetticolum*

Varieties tested	Unpunctured					Punctured				
	Number of tests	Total shoots	Per cent positive	Per cent doubtful	Per cent negative	Number of tests	Total shoots	Per cent positive	Per cent doubtful	Per cent negative
LIMES										
West Indian	23	74	57	11	32	39	109	93	4	3
Dominican Thornless	3	12	100	0	0	5	19	100	0	0
Persian	4	13	0	5	95	11	28	0	18	82
Kusaie	4	24	0	0	100	13	70	0	0	100
Sour Lime No. 7338 [a]	2	6	0	0	100	8	19	0	0	100
Sweet	3	19	0	0	100	4	26	0	4	96
Rangpur	9	41	0	2	98	19	80	0	4	96
LIME HYBRIDS										
Limequat	7	11	0	9	91	8	18	0	11	89
Faustrime	19	118	0	0	100	42	302	0	9	91
CITRONS										
Citrus medica (Susceptible strains)	14	31	0	13	87	34	87	23	21	56
Citrus medica (other strains)	17	35	0	0	100	38	84	0	20	80
Sour Citron No. 7425 [a]	5	9	0	22	78	6	25	0	8	92
Etrog Citron	3	10	0	0	100	9	20	0	5	95

[a] Serial numbers of the Office of Crop Physiology and Breeding Investigations Bureau of Plant Industry, U. S. Department of Agriculture. The writer acknowledges his indebtedness to this office for a large number of the unusual varieti[illegible] included in the present investigation.

TABLE II.—*Greenhouse tests by artificial inoculation for susceptibility to Gloeosporium limetticolum*—Continued

Varieties tested	Unpunctured					Punctured				
	Number of tests	Total shoots	Per cent positive	Per cent doubtful	Per cent negative	Number of tests	Total shoots	Per cent positive	Per cent doubtful	Per cent negative
LEMONS										
Eureka	1	1	0	0	100	2	6	0	0	100
Lisbon						1	2	0	0	100
California Seedling	4	5	0	20	80	8	15	0	20	80
Villa Franca	1	1	0	0	100	3	6	0	33	67
Kenedy	1	2	0	0	100	2	8	0	0	100
Dwarf Chinese	4	9	0	11	89	11	16	0	6	94
Ponderosa	3	5	0	0	100	12	44	0	5	95
Malta Lemon	3	4	0	0	100	6	13	0	8	92
Lemon sp.	4	8	0	0	100	6	13	0	34	66
GRAPEFRUIT										
Marsh	3	19	0	0	100	3	19	0	0	100
Silver Cluster	1	3	0	0	100	3	11	0	0	100
Gold Medal	1	3	0	0	100	1	5	0	0	100
Chinese Pummelo No. 11213 [a]	2	6	0	0	100	5	15	0	7	93
Sour Pomelo No. 7440 [a]	1	1	0	0	100	6	9	0	11	89
Grapefruit seedlings	7	22	0	0	100	20	74	0	9	91
ORANGES										
Valencia	2	12	0	0	100	1	6	0	0	100
Pineapple	2	4	0	0	100	4	11	0	0	100
Parson Brown	3	16	0	0	100	3	16	0	0	100
Mediterranean Sweet	2	18	0	0	100	3	14	0	0	100
Ruby Blood	3	20	0	0	100	7	18	0	0	100
China Sweet	3	7	0	0	100	5	12	0	8	92
Golden Ring	1	5	0	0	100	2	10	0	0	100
Miscellaneous	1	1	0	0	100	17	41	0	2	98
Florida Seedling	2	18	0	0	100	4	14	0	0	100
MANDARIN GROUP										
Tangerine	2	6	0	0	100	6	15	0	0	100
Mandarin	2	15	0	0	100	8	34	0	0	100
Satsuma	4	11	0	0	100	7	17	0	0	100
King						1	6	0	0	100
Temple						1	10	0	0	100
Clematine	1	5	0	0	100	3	7	0	0	100
C. nobilis	1	3	0	0	100	4	9	0	0	100
VARIOUS HYBRIDS										
Faustrimedin	3	25	0	0	100	5	38	0	5	95
Tangelo varieties	11	49	0	0	100	23	78	0	10	90
Orangelo varieties	1	5	0	0	100	3	11	0	0	100
Citrange varieties	1	7	0	0	100	4	7	0	0	100
Miscellaneous seedling hybrids	1	6	0	0	100	7	28	0	7	93
VARIOUS CITRUS										
Sour orange	2	4	0	0	100	4	17	0	0	100
Bittersweet orange	4	4	0	0	100	8	14	0	0	100
Rough lemon	2	7	0	0	100	4	21	0	0	100
Myrtle-leaved orange						1	2	0	0	100
Citrus sp. Mamis	2	2	0	0	100	3	10	0	10	90
Citrus Caibe or Moi	1	2	0	0	100	4	7	0	0	100
Limon Real						3	4	0	0	100
Citrus mitis	6	14	0	0	100	16	47	0	2	98
Citrus excelsa	9	25	0	0	100	16	40	0	5	95
Citrus hystrix	4	8	0	0	100	4	18	0	6	94
Otaheite orange	4	11	0	0	100	9	43	0	2	98
Double Flowering orange	1	2	0	0	100	2	12	0	0	100
Saigon orange	1	1	0	0	100	5	8	0	12	88
Ladoo orange	3	13	0	0	100	3	7	0	0	100
Kansu orange	3	4	0	0	100	5	14	0	0	100
Ichang lemon	5	14	0	0	100	19	53	0	15	85
RELATED GENERA										
Poncirus trifoliata	2	4	0	0	100	4	10	0	0	100
Nagami kumquat	1	1	0	0	100	8	19	0	16	84
Fortunella crassifolia	3	7	0	0	100	8	30	0	0	100
Microcitrus australis	2	13	0	0	100	8	52	0	6	94
Chaetospermum glutinosum	2	2	0	0	100	5	16	0	0	100

[a] Serial number of the Office of Crop Physiology and Breeding Investigations Bureau of Plant Industry, U. S. Department of Agriculture. The writer acknowledges his indebtedness to this office for a large number of the unusual varieties included in the present investigation.

The cases recorded above as doubtful showed decided death of tissue, but did not develop typical *Gloeosporium limetticolum* sporulation. It is very likely that such lesions were produced by saprophytic organisms that were able to invade and break down tissues under the rather extreme conditions of inoculation. In the writer's opinion these doubtful cases are in all probability negative.

In the case of the citrons, 10 strains or varieties were furnished by the Office of Crop Physiology and Breeding Investigations. Six of these lots did not show any positive evidence of infection by *G. limetticolum*, and they are averaged together in the tabulation. Four other lots did show low percentages of what seemed to be positive, although not perfectly typical infection. This was verified by culturing *G. limetticolum* from the lesions on punctured shoots, and by securing typical infection on limes from such cultures; and on this basis a positive record was made in such cases. However, the absence of any such cases on the unpunctured shoots, and the occurrence on citrus of a large proportion of doubtful lesions in which the presence of *G. limetticolum* could not be demonstrated, would lend support to the view that tender shoots of these citrons are rather subject, under the conditions of the puncture experiments, to invasion by saprophytic fungi among which *G. limetticolum* may sometimes be found. Certainly these citrons, classed as susceptible, are affected with far less frequency than the susceptible varieties of limes, and the lesions recorded as positive did not have all the typical features of lime wither tip.

Besides these somewhat questionable results from certain citrons, there is no positive susceptibility except in the group of limes, and among the varieties tested in this group the susceptibility is sharply confined to the West Indian lime and to Dominican Thornless lime.

GENERAL DISCUSSION

Clausen,[7] in his description of *G. limetticolum*, gives three host plants, *Citrus medica* var. *acida* Hook. (=*C. aurantifolia* Sw., and includes the West Indian lime which is referred to by Clausen as sour lime), *C. limetta* Risso, and *C. limonis* L. Clausen explains that the last two were artificially infected in greenhouses and that "the report of pathogenic properties on the sweet lime, *C. media* var. *limetta*, is according to C. O. Smith who is working at Whittier, Calif., with a strain of fungus supplied by the writer." The strain of Sweet lime used in the present investigation did not give positive results in 7 tests including 45 shoots, punctured and unpunctured as well as in 2 special tests of 3 plants each. Clausen reports only 2 tests of lemons (*C. limonis* L.=*C. limonia* Osb.) with *G. limetticolum*. Two trees were used in each; 1 test was negative for both trees, in the other test one tree developed "an infection of three young leaves, the other lemon, as well as the control, was not affected in any way." He further states, "the extent of parasitism on the lemon was not fully demonstrated in the experiments, but the parasitism in the one positive case was unmistakable." Clausen's inoculation tests included: 19 on sour (West Indian) lime of which 13 were positive, 4 on lemon of which 3 were negative, 8 negative on sweet orange, 8 negative on sour orange, 7 negative on grapefruit, and 4 negative on tangerine orange. Adopting the view that the sour lime, the sweet lime, and the lemon are all varieties of *C. medica* L., he states in his summary that the wither-tip fungus in his artificial infection attacked only forms and varieties of this one species.

The present investigation gives much more extensive evidence that most citrus types are immune to attack by *G. limetticolum*. It casts doubt on the reported susceptibility of sweet lime and lemon, and raises the question of whether or not certain citrons may have slight susceptibility when wounded.

It is interesting to note that commercial plantings of the West Indian lime are commonly made from seedlings. These have remarkable similarity in their general characteristics, and all seem to be highly susceptible to the wither-tip disease. A constant lookout has been kept in affected groves, and special tests of seedlings have been made for individuals immune to the disease. As yet no such individual has been found. It is possible that such individuals do exist, and the prompt discovery of one plant with high resistance or complete immunity would mean a quicker and more satisfactory solution of the wither-tip-control problem than the substitution of some other citrus variety that is immune, or the development of some new type by hybridizing.

[7] CLAUSEN, R. E. Op. cit. p. 232.

The Dominican Thornless lime seems almost as susceptible as the ordinary West Indian lime. The percentages of experimental infection were very much the same for these two varieties; the experimental lesions on the former usually developed more rapidly and extensively than on the latter; but the natural grove infection seemed somewhat less severe. This thornless type, commonly supposed to be a sport from the West Indian lime, is quite distinct from it in fruit, leaf, and stem characteristics. Seedlings from it seem to be more variable than those from the West Indian lime. Keys [8] has reported a peculiar dying and shedding of the flowers and very young pistils, due to an aborted condition of the stigmas, which might be confused with wither-tip attack.

Is susceptibility to attack by *G. limetticolum* a dominant character in inheritance? The practically universal susceptibility of West Indian lime seedlings would support a positive answer if the embryos result from true fertilization. On the other hand, the two hybrids tested that have West Indian lime parentage are nonsusceptible. Certainly the Dominican Thornless lime is very susceptible, and is the only citrus variety besides the West Indian lime out of several scores in the present tests for which there is unquestioned evidence of high susceptibility. It will be interesting and important from the standpoint of genetics to test the susceptibility of a considerable number of seedlings of the Dominican Thornless lime, as well as more hybrids of the West Indian lime.

SUMMARY AND CONCLUSIONS

The evidence here presented includes field observations on a large number of citrus varieties, species, and related genera growing near lime trees that were always severely infected with lime wither tip, as well as the results of artificial inoculations made during three seasons on wounded and on unwounded tender shoots both in groves in Florida and in a greenhouse near Washington, D. C. The West Indian lime and the Dominican Thornless lime have constantly shown a high degree of susceptibility to infection by *Gloeosporium limetticolum* of very young leaf, stem, and fruit tissue. These two varieties are probably closely related to each other. None of the other varieties of limes tested has given undoubted indication of susceptibility. Certain strains of citron (*Citrus medica* L.) have shown atypical invasion of wounded tissue by *G. limetticolum* in a comparatively small percentage of cases under greenhouse inoculation. Other varieties of limes (*C. aurantifolia* Sw.) as well as the majority of varieties of *C. medica*, have been absolutely immune to infection under the very severe conditions of the tests. This also holds true for certain first-generation hybrids having West Indian lime parentage that were subjected to test, and for representative varieties of round orange (*C. sinensis* Osb.), for grapefruit (*C. grandis* Osb.), for lemon (*C. limonia* Osb.), for kid-glove oranges (*C. nobilis* var.), and for a considerable number of miscellaneous species of Citrus and genera related to Citrus.

The close restriction of susceptibility in so far as is known practically to two very closely related seedling horticultural varieties of lime gives promise for successful substitution of other types of lime for the susceptible ones as a means of ultimately escaping losses from the disease, and points to the availability of a wide range of immune breeding stock as a basis for hybridization. Susceptibility seems not to be a dominant character in first-generation hybrids with other citrus species, but the natural seedlings of the West Indian lime, practically without exception, are highly susceptible. However, there is the possibility that a natural seedling may be found possessing immunity and having the desired characters of the West Indian lime. Such a find would possibly result in most effective and satisfactory control of the very destructive lime wither-tip disease.

[8] [KEYS, A.] ABNORMAL FLOWERS OF THE SPINELESS LIME. Rept. Agr. Dept. Dominica [Brit. West Indies] 1922–23: 23–24. 1923.

REFORESTATION BY SEED SOWING IN THE NORTHERN ROCKY MOUNTAINS [1]

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INTRODUCTION

Planting denuded forest lands with nursery stock is the dependable and established way to get new forest crops started. The fact, however, that it is in many places an operation involving heavy expenditures has led the American forester to try out other methods to a considerable extent. His characteristic discontent with established practice as such, and his constant and persistent efforts to discover better ways, have led him to give not a little attention to field sowing as a possible short-cut in the artificial regeneration of forests. He considers that it is certainly successful under many conditions in nature, and he is disinclined to abandon the idea, even under very adverse conditions, because the procedure appears to be simple and close to nature's plan. When seeds germinate and the plants grow to maturity undisturbed by man there is at any rate the certainty that the root system will be normal and that the trees will not be unduly subject to windfall or similar dangers which might result from planting.

Such attempts at direct seeding in the northern Rocky Mountain region fall into two distinct classes: (1) The early extensive projects and (2) the later intensive experiments.

EXTENSIVE EXPERIMENTS

In 1910, large and very destructive fires occurred throughout the Rocky Mountain region, and the earnest desire to get new forest crops started led to extensive direct seeding projects during the following years. In all, 2,899 acres were broadcast, 10,511 acres were sown by means of corn planters, and 1,969 acres were sown in spots—a total of 15,379 acres on the national forests in the western mountains. Of this area, 53 per cent was seeded to western white pine (*Pinus monticola*), 36 per cent to western yellow pine (*P. ponderosa*), 5 per cent to Douglas fir (*Pseudotsuga taxifolia*), and 6 per cent to other species, such as lodgepole pine (*Pinus contorta*), Engelmann spruce (*Picea engelmanni*), Norway spruce (*P. excelsa*), western larch (*Larix occidentalis*), limber pine (*Pinus flexilis*), and hardwoods. Most of these areas have been examined at least once since sowing.

Out of 343 trials, only 20 succeeded. Failures were found to be absolute in practically all cases, and all areas found to have less than 100 trees per acre were classed as failures. Broadcasting was tried generally without preparation of soil or use of poison. Of 101 attempts to reforest by this method, all failed except 9. The 153 attempts to reforest by the use of a corn planter failed likewise, with 9 exceptions. Of 89 attempts to use the seed-spot method, only 2 were successful.[2]

There is one notable success, however, on the Lolo National Forest. The ranger who made this sowing attributes the exceptional success to four things: (1) Prompt sowing after a burn; (2) seed poisoned with red lead and the area strewn with poisoned oats; (3) favorable sites; and (4) a moist season. In this case the sowing cost $4.46 per acre for labor. Other expenses brought the total cost to $6.31 per acre.

Failure is the outstanding fact in the vast majority of cases. It would be desirable, if possible, to look more closely into the reasons for the few instances of success, for the causes which made possible these exceptions might furnish some data for further observation. Unfortunately, recorded information is lacking concerning details of sowing, site, or seed conditions, some of which may have been vitally important.

[1] Received for publication June 23, 1924; issued June, 1925.

[2] In the cases both of broadcast and spot plantings there was one additional trial which appeared successful at the end of the first season but has not been examined since.

Experiments in direct seeding carried on at the Priest River branch experiment station in northern Idaho led conclusively to the opinion in 1916 that broadcast and corn-planter sowing were in general unreliable and too expensive. Since then the seeding experiments of the Forest Service in this region have been confined to sowing in prepared spots.

INTENSIVE EXPERIMENTS

In western Montana the Forest Service has had detailed seed-spot experiments under way since the spring of 1916 as a part of the planting research work at Savenac Nursery. As a preliminary step, the comparison of the adaptability of different species to seeding methods was undertaken. For this purpose it was necessary to group the species on the same site, the plan being to make later tests of the promising species on sites well adapted to each. The location selected near Haugan, Mont., is typical of the areas in need of reforestation in the region. The sites used had been severely denuded by fire in 1910, only a few snags having been left standing. Fallen trees were partly consumed by fire. The aspects are north and northwest, and the elevation ranges from 3,500 to 4,500 feet. The slopes are 35 to 65 per cent, averaging about 50 per cent, and, aside from a mixture of herbaceous plants, are being reclaimed by occasional willows (Salix), buckbrush (Ceanothus), and flowering raspberry (Rubus). The soil is a loam, clayey and stony in places.

On these areas, seed spots were installed every spring from 1916 to 1921, and during the fall in 1916 and 1918. Thirty tests consisting of 300 prepared spots each were installed during the six-year period.

A method of preparing and sowing spots which seemed to give most promise of success was adopted as standard and adhered to throughout, except in the case of sample lots of seed spots treated differently to give a check on methods. The standard method was as follows:

A surface 6 to 8 inches square was denuded of sod and herbaceous cover by means of a planting mattock. Twenty to twenty-five seeds were scattered on the resulting fresh soil surface and tamped into this soil by pressing with the flat of the mattock blade. The seeds were then hand-covered by scattering on loose soil to the desired depth, or approximately $\frac{3}{8}$ inch for western yellow pine, $\frac{1}{4}$ inch for Douglas fir and Engelmann spruce, and $\frac{1}{8}$ inch for western red cedar (*Thuja plicata*). The soil cover was not tamped.

The variations of this method included tamping the surface soil, soil sterilization against fungi by a treatment with sulphuric acid, covering with leaf litter to make spots less conspicuous to rodents and birds, and screening against these enemies.

Observations were made and recorded every 7 to 10 days during the first two growing seasons, with spring and fall survival counts the third season. The records at 10-day intervals showed germination, loss by causes, and survival. Seedlings were marked individually at the time of germination with toothpicks stained a distinctive color for each month. Subsequent notes on seedlings were recorded according to month of germination.

In 1916 western yellow pine failed because of the activity of rodents, although the survival of the seedlings, once germinated, was the highest of all species. Western red cedar failed because of low germination and drought, rodents being of no importance. The Douglas fir sowing was only a partial success, for most of the seed was damaged by rodents previous to germination, although the percentage of survival was high. Engelmann spruce, although it lost more than one-third of the plants from various causes, principally drought and cutworms, and to some extent damping-off, had survivors in three-quarters of the unprotected spots averaging more than three per spot.

In order of importance the causes of loss during 1916 were drought, insects, and fungi. The sulphuric-acid treatment did not materially lessen damping-off. The expense of using a litter cover to mask the spots against rodents did not appear to be justified, for it had but slight effect. Rodents interfered only with the large seeds. The conclusion of the season was that the work gave promise of success and justified further attempts.

In 1917 the sowings of 1916–17 were watched closely. Douglas fir proved to be not much troubled by rodents and nearly as drought resistant as the western yellow pine. Of this species, 50 per cent of the spots sown in the spring and 22 per cent of those sown in the fall of 1916 had survivors in the fall of 1917. Western larch was second with survivors in 11 per cent of fall-sown and 3 per cent of spring-sown spots. Yellow pine and Engelmann

spruce were reduced to 6 per cent and 3 per cent, respectively, in the fall series, and to zero in the spring of 1917 series. The western yellow pine proved to be very drought resistant, but both series were complete failures because of the rodents. Western white pine and red cedar were also complete failures, largely because of drought.

The conclusions of the 1917 season were similar to those of a year before but were less optimistic. The practice of seed spotting appears at best an uncertainty, much more so than planting. The results obtained certainly indicated the advisability of giving up attempts at seed spotting and of placing all attention on the surer method of planting. But in view of the greater amount of work done on planting experiments in comparison with seed spots, it was thought that the work to date warranted further attempts with Douglas fir and, to some extent, with Engelmann spruce.

The season of 1918 was more favorable. The examinations made at 7 to 10 day intervals showed a loss from drought of approximately 50 per cent in Engelmann spruce and larch. The larch was the least resistant to harmful agencies, mainly drought and fungi. Douglas fir was decidedly superior to the others, showing a survival of 58 per cent at the close of the season.

During the following season the sowings of spring and fall of 1918 and spring of 1919 were watched. The superiority of Douglas fir appeared in each case. Western white pine was next in the fall sowings, and larch and spruce were last with but 5 per cent survival in 2-year-old spots. The spring sowings of 1919 succumbed to drought. Because of the extreme dryness of the season, drought was the foremost cause of loss.

In 1920, observations of all sowings, from the spring of 1918 to those of the spring of 1920, showed Douglas fir to be superior without exception.

In 1921, the survivals from previous series were still decreasing perceptibly in numbers. That season's sowings failed completely because of drought and cutworms.

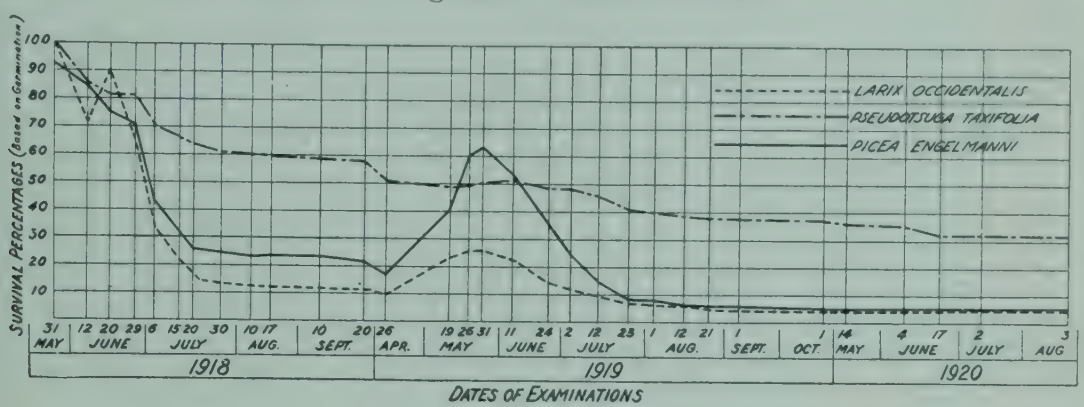
SURVIVAL PERCENTAGES (based on Germination)
LARIX OCCIDENTALIS
PSEUDOTSUGA TAXIFOLIA
PICEA ENGELMANNI
31 MAY | 12 20 29 JUNE | 6 15 20 30 JULY | 10 17 AUG. | 10 20 SEPT. | 26 APR. | 19 26 31 MAY | 11 24 JUNE | 2 12 23 JULY | 1 12 21 AUG. | 1 SEPT. | 1 OCT. | 14 MAY | 4 17 JUNE | 2 JULY | 3 AUG
1918
1919
1920
DATES OF EXAMINATIONS

FIG. 1.—Series sown, spring of 1918

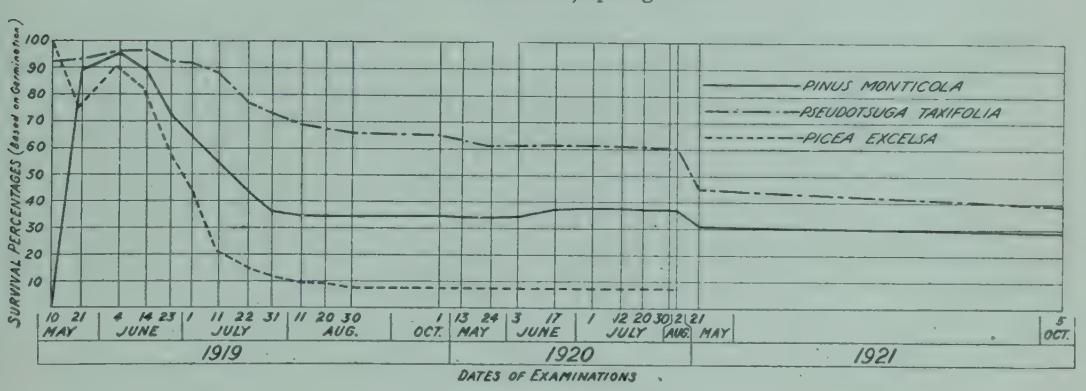
SURVIVAL PERCENTAGES (based on Germination)
PINUS MONTICOLA
PSEUDOTSUGA TAXIFOLIA
PICEA EXCELSA
10 21 MAY | 4 14 23 JUNE | 1 11 22 31 JULY | 11 20 30 AUG. | 1 OCT. | 13 24 MAY | 3 17 JUNE | 1 12 20 30 JULY | 2 AUG. | 21 MAY | 5 OCT.
1919
1920
1921
DATES OF EXAMINATIONS

FIG. 2.—Series sown, fall of 1918

RESULTS

What has been the result as a whole of the six years of painstaking effort? What percentage of the total number of spots now contain one or more survivors? The best record is about 20 per cent for Douglas fir. Other species are not above 15 per cent in any case.

The marked superiority of Douglas fir in every sowing is evident. Although its germination as compared with its associates was less in most cases, fewer of the seedlings of this species were lost; and in every case the last examination showed Douglas fir to have more survivals per spot and a larger portion of spots with survivals.

The application of a covering of litter to mask the spots against the raids of rodents, and the use of sulphuric acid as a fungicide were ineffective. The results did not justify the expense of these operations.

In general more deaths were due to drought than to any other two causes. (Table I.) During dry summers, which have been frequent in this region of late years, the soil easily dries out completely to a greater depth than the roots of the very young seedlings have been able to penetrate. A high evaporation rate occurring at the same time soon kills them. Cutworms were next in destructiveness. Fungi were not a large factor. The remaining classes of loss, "accident," and "missing," represent numerous deaths from miscellaneous causes. Frost heaving is often an important cause of loss.

shrubs filled some of the spots and were pressed into a solid mat by snow, thus forming a mechanical barrier to germination the next spring.

Yellow pine was early abandoned in this work because of the abundance of rodents, which seek especially these large seeds. On the experimental area the western chipmunk (*Tamias quadrivitatus*) and a similar animal, Say's spermophile (*Spermophilus lateralis*), have been observed to fairly "swarm" in August and early September, while in late October one is rarely seen. Perhaps sowing should be done late, even at a sacrifice of some germination, in order to avoid them. It is likely that one of the principal reasons for the success of restocking on certain areas by broadcast seeding after the 1910 fires was the wholesale destruction of rodents by the fire.

TABLE I.—*Causes of loss of seedlings in seed spots, Haugan experiment area, Trail Gulch*

Species	Row No.	Date of sowing	Years considered	Percentage of loss from various causes						
				Fungi	Cutworms	Drought	Accident [a]	Missing	Other	Total
Picea Engelmanni	1	Apr. 26, 1918	1918–1920	7.9	15.7	64.1	1.8	7.2	3.3	100
Larix occidentalis	2	Apr. 27, 1918	1918–1920	17	9.2	59	1.5	9.3	4.0	100
Psd. taxifolia	3	Apr. 29, 1918	1918–1920	22.2	14.4	34.3	.9	10	18.2	100
Pinus monticola	4	Sept. 11, 1918	1919–1921	2.2	30.9	52.9	.7	11.8	1.5	100
Psd. taxifolia	5	Sept. 27, 1918	1919–1921	0	9.3	49.5	6.2	35.1	0	100
Larix occidentalis	6	Sept. 28, 1918	1919–1921	3.9	12.8	62	4.2	14.3	2.9	100
Picea excelsa	7	Oct. 3, 1918	1919–1920	2	22	71	1	4	0	100
Picea Engelmanni	8	Oct. 2, 1918	1919–1920	2.9	14.8	75.2	1.6	4.2	1.3	100
Do	9	Oct. 4, 1918	1919–1920	3.5	20.1	67.3	2.1	5.8	1.2	100
Psd. taxifolia	10	May 10, 1919	1919–1921	10.3	18.3	55.9	0	15.6	0	100
Picea Engelmanni	11	May 22, 1919	1919–1921	5.7	17.1	56.5	.4	20.2	.1	100
Pinus monticola	12	Sept. 11, 1919	1919–1921	2.6	25.7	44.7	5.9	21.1	0	100
Larix occidentalis	13	Sept. 12, 1919	1920–1921	11.1	14.6	24.6	22.7	27	0	100
Psd. taxifolia	14	Oct. 6, 1919	1920–1921	3	30.2	20.7	16	30.2	0	100
Picea Engelmanni	15	Oct. 7, 1919	1920–1921	6.4	32.2	27.1	13.6	20.8	0	100
Larix occidentalis	16	June 2, 1920	1920–1921	2.7	10.8	8.1	18.9	59.4	.1	100
Picea Engelmanni	17	June 3, 1920	1920–1921	1.7	22.4	38.7	12.5	24.6	.1	100
Psd. taxifolia	18	June 4, 1920	1920–1921	8.5	21.3	17	34	19.3	0	100
Picea Engelmanni	19	May 5, 1921	1921	5.2	51.9	30.8	1.1	11.1	0	100
Larix occidentalis	20	----do----	1921	4.8	44.4	29.1	.5	21.1	.1	100

[a] Among the miscellaneous causes of loss listed as accident, frost heaving seems to have been the most important.

Stones in seed spots and lying near the sowing surface presented mechanical obstacles to seedlings starting on the thin layers of soil. When the life of a seedling depends upon the deep penetration of its root before the dry summer season arrives, such obstacles are often fatal. Stones lying on the surface become very hot in the sun, attaining temperatures injurious to growing tissue; these possibly caused the death of seedlings next to them in some instances. In other cases the seed spots formed little pockets on the slope which caught rolling stones and débris. Leaves from hardwood

The tendency for western white pine seed to reserve a large part of its germination until the second season after sowing is well known. Contrary to expectations, Engelmann spruce twice exhibited such delayed germination. Both times the occurrence was in series sown in the spring, whereas fall sowings were entirely free from such hold-over germinations.

There was a complete loss before August of all germinations in screened spots sown in 1921. This is apt to happen to seed spots in this region when a dry year follows sowing. Survival data show that in most cases,

although the remaining seedlings are approaching establishment, they are still decreasing in numbers.

In this connection a statement made by a German forester since the recent war is interesting. Conditions in Germany, of course, are different from ours, and conclusions from work elsewhere can not be adopted as they stand. Nevertheless, it is well to keep in mind the development in a country older in forestry than our own. Kienitz [3] concludes that "... natural regeneration has its rightful sphere as has sowing and planting; but, in general, artificial culture has progressed far in advance and now is passing out of the sowing stage into that of the higher forest industry, namely, planting." Germany has found direct seeding successful only when a continuous moisture supply is available. The short superficial root system of first-year seedlings makes them succumb easily to drought. Planting results in a better distribution of soil moisture about the roots because of the loosened soil in the planting holes. It is the preferred method, and German experiments have shown that the cost of planting, calculated through the first three years, is less than the cost of sowing.

CONCLUSIONS

Of all the early direct-seeding projects in the northern Rocky Mountain region, only 6 per cent were successful or partially successful. Later intensive experiments with seeding in prepared spots also failed to indicate practicable methods of direct seeding for this region. Douglas fir was found to be much better adapted to success than the other local species, but all trials failed to produce a stand of survivors in sufficient numbers. Because of rodents most of the large seed sown was never allowed to germinate. Among the seedlings drought was the foremost cause of loss; cutworms were next in destructiveness, and frost heaving caused many deaths. Fungus trouble was experienced, but ordinarily was not severe. Speaking in terms of the percentage of spots with one or more survivors, about 20 per cent survival was obtained with Douglas fir under the best conditions, whereas other species were below 15 per cent in survival in all cases. Western white and western yellow pine and Engelmann spruce, important commercial timber trees of the region, are essential in reforestation and are included in the group of species making an extremely poor showing in the experiments.

It may be that direct seeding has its rightful sphere in the northern Rocky Mountain region, but if so, it has not yet been discovered. On the basis of the results here recounted, no more experiments in direct seeding are contemplated, except a test of sowing in fresh ashes after fires. A little hope for this method is cherished, but, if it succeeds, application in the region would necessarily be restricted to sites free from rodents, which possibly can be found only in the center of large, newly burned areas.

The only hope seems to lie in man's ability to reproduce the conditions which make possible nature's own direct seeding, or at least to recognize and approximate them. Nature, however, sows her seed more lavishly than the forester can afford to in his work, even to the extent of several hundred thousand large seeds and several million smaller seeds per acre, in order to secure a stand of 5,000 seedlings.[4] And she is more patient with her many delays and failures than the forester can afford to be with his.

[3] KIENITZ, M. WAS IST DENN JETZT MODE: SAAT ODER PFLANZUNG? Ztschr. Forst u. Jagdw. 51: 417–436. 1919.

[4] Based on careful counts made in the region by J. A. Larsen.

TWO IMPORTED EGG PARASITES OF THE GIPSY MOTH, ANASTATUS BIFASCIATUS FONSC. AND SCHEDIUS KUVANAE HOWARD [1]

By S. S. Crossman

Entomologist, Gipsy Moth and Brown-Tail Moth Investigations, Bureau of Entomology, United States Department of Agriculture [2]

INTRODUCTION

The State of Massachusetts, cooperating with the United States Department of Agriculture, began in 1905 to introduce into the United States foreign insect enemies of the gipsy moth (*Porthetria dispar* L.) and the brown-tail moth (*Euproctis chrysorrhoea* L.). This arrangement continued through 1911, when the Bureau of Entomology, United States Department of Agriculture, took over all of the parasite work.

Among the several foreign parasites of the gipsy moth which have been established in the United States are two species which develop within the egg of the moth. These species, known as *Anastatus bifasciatus* Fonsc. and *Schedius kuvanae* How., are now well established and both are of considerable importance as enemies of the gipsy moth in New England.

Schedius kuvanae is a native of Japan. When it was first found in gipsy-moth eggs received from Japan it was sent to L. O. Howard, who found it to be an undescribed species and genus of the Encyrtidae. *Anastatus bifasciatus*, also a member of the family Encyrtidae, has a much wider distribution, occurring in many parts of Europe, as well as in Japan.

IMPORTATION OF ANASTATUS BIFASCIATUS AND SCHEDIUS KUVANAE

The only method attempted in introducing these species was to ship the host eggs from their native lands to the United States. The masses of eggs of the gipsy moth were received packed loosely in small boxes and in cloth bags inclosed in wooden boxes. Many of the egg masses from Japan arrived wrapped separately in rice paper and packed in layers between blotting paper. Some of the shipments came in cold storage, but many were sent as ordinary express packages or by mail. From 1906 until the spring of 1910 the gipsy moth laboratory at Melrose Highlands, Mass., received many such clusters from Japan and from several countries in Europe.

When the egg masses are collected and shipped in the fall, cold storage is not necessary, since Anastatus remains in the host egg normally about 11 months. In the case of Schedius, which produces several generations during the fall, adults sometimes issue en route and parasitize some of the host eggs in the containers, thus carrying the species through to its destination. Later collections of egg clusters which have been exposed to low temperatures should be sent in cold storage, for if they are exposed to high temperatures the development of the parasites and the gipsy moth larvae is accelerated too much and may result in the issuance of the adult parasites and the hatching of the eggs.

A few gipsy-moth eggs received in 1906 from Basel, Switzerland, contained no parasites. In 1907 several small lots of egg clusters were received from Japan and Russia. No parasites were recovered from the Russian material, but one of the lots of eggs from Japan, although badly broken, showed evidence of parasitism.

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[1] Received for publication Apr. 22, 1924; issued June, 1925.

[2] A. F. Burgess has been in direct charge of the parasite work of the gipsy moth laboratory, Bureau of Entomology, U. S. Department of Agriculture, since 1912. It would be difficult to name all those associated with the laboratory who have at one time or another been connected with the work. W. F. Fiske and H. S. Smith, assisted by R. Wooldridge, did much of the first investigational work with these parasites. Since then C. W. Stockwell, D. W. Jones, and J. A. Millar have greatly developed and improved the apparatus used in this work, and thus made it possible to handle large amounts of gipsy-moth eggs and parasites with a minimum amount of hand labor. The photographs illustrating this article were made by W. N. Dovener and C. E. Hood.

During the entire period L. O. Howard, Chief of the Bureau, has had supervision of the work. Many foreign entomologists have contributed to the parasite introduction project, and without their interest and aid much of the work would have been impossible.

In 1908 about 800 egg clusters came from Kief, Kishenef, and Simferopol, Russia; from those from the two places last named 470 individuals of Anastatus were recovered. A few egg clusters received from Charroux, France, showed no evidence of parasitism. During that year many attempts were made to introduce the egg parasites from Japan. Fourteen separate shipments were made, and although many dead Schedius and over 5,000 living individuals of Anastatus were received, it was not until December, 1908, that a single living Schedius was obtained. This individual was a male which died before a female was reared.

In 1909 shipments of eggs continued in larger numbers than previously, egg masses being received from Japan, Russia, Hungary, and Rumania. Nearly 10 quarts of egg masses arrived from Hungary, and from some of these the majority of the Anastatus were obtained. Anastatus was recovered from the shipments of all the countries except Rumania. In 1909 a total of 5,714 living individuals of Schedius were reared from the gipsy moth eggs which came from Japan.

In 1910 egg clusters were received from Japan, France, Germany, and Russia, but in smaller numbers than during the previous year. Living Schedius and Anastatus were recovered in small numbers from the Japanese importations, and Anastatus was present in eggs from each of the European countries from which eggs were received.

DISTRIBUTION OF A. BIFASCIATUS AND S. KUVANAE IN JAPAN AND OF A. BIFASCIATUS IN EUROPE

Schedius kuvanae and *Anastatus bifasciatus* have been reared at the gipsy moth laboratory from eggs of the gipsy moth which were collected in Japan from the towns of Akabane, Fukuoka, Funakimura, Nishigahara, and Tokyo.

Anastatus bifasciatus has been recovered from its host's eggs received from the following places in Europe: Crimea, Schiriya, Kief, and Kishenef, Russia; Lippa (Temes), Huszt (Maramoras), Dorgas (Temes), and Sistarobecz (Temes), Hungary;[3] Schlesien, Germany, and Nantes, France.

In 1922 and 1923 the writer examined eggs of the gipsy moth in France, Spain, Italy, Sicily, Germany, and Hungary. Anastatus was found in small numbers in eggs at Hyères, (Var), France, Madrid, Spain, Dahlem, Germany, and Caltagirone, Sicily.

The examination of the eggs of the gipsy moth in the field and data obtained from examinations at the gipsy moth laboratory at Melrose Highlands, Mass., show that Anastatus is a widely distributed species, but that Schedius is present in Japan only. Both species in their native lands are locally distributed and many of the egg clusters, collected from different locations, have been received at Melrose Highlands which showed no evidence of parasitism.

VALUE OF GIPSY-MOTH EGG PARASITES IN JAPAN AND EUROPE

The data obtained from examination of large numbers of gipsy-moth eggs from Japan and Europe show that both parasites discussed in this article are of considerable value as enemies of the gipsy moth in their native countries.

It would appear from the examination of eggs received from Japan, most of which were sent by S. I. Kuwana, of the Imperial Agricultural Station at Tokyo, and from observations made by J. N. Summers, of the Bureau of Entomology, that *Schedius kuvanae* is more common in Japan than is *Anastatus bifasciatus*. The two species often were present in eggs collected in the same localities, but in no case was the percentage of parasitism by both species high under these conditions. A number of collections showed no parasitism. Other collections gave as high as 33.3 per cent parasitism by Schedius. The parasitism by Anastatus usually ran lower than that caused by Schedius, and no collections were received which showed more than 2 or 3 per cent parasitism, although examination of individual egg clusters ran as high as 15 per cent.

The parasitism of eggs of the gipsy moth in Europe is very variable. Many collections from Europe received at Melrose showed no parasitism. The parasitism of eggs collected from points where Anastatus was present ran from 1 per cent to 25 per cent. The highest percentage of parasitism was obtained from a large shipment of eggs from the southeastern part of Hungary (now in Rumania), in which some 80,000 parasites were recovered. This is evidence of the value of *Anastatus bifasciatus* in parts of Europe during the season in which the eggs were collected.

[3] These towns were in Hungary before the World War. They are now a part of Rumania, except Huszt, which is in Poland.

During the last two or three years the Service for the Study and Extinction of Forest Plagues in Spain has been utilizing this parasite in its control work against the gipsy moth by transferring Anastatus from areas where it is abundant to the areas where it is not present.

EARLY EXPERIMENTS IN REPRODUCTION TO OBTAIN SCHEDIUS KUVANAE FOR COLONIZATION

In December, 1908, the first living Schedius was reared from Japanese gipsy moth eggs at the gipsy-moth laboratory. This was a male which soon died. Early in January, 1909, a female Schedius was reared. This female reproduced parthenogenetically, and 15 male Schedius were reared in February from the eggs in which she had oviposited. An attempt to mate her with her progeny was unsuccessful and she died without further offspring. In April, 1909, 12 adult Schedius were reared from Japanese eggs and used in starting several series of reproduction experiments. One of the females died soon after the beginning of the experiment. One hundred adult Schedius were obtained through these experiments, the first generation to be developed in America. Added to these were 21 adults which issued from imported eggs. The second generation gave 643 adults, which in turn produced 1,350 Schedius in the third generation. To these Schedius of the third generation were added 1,671 adults from imported eggs. These reproduction experiments, started in April, continued throughout the year 1909. In August, after the progeny from the fourth generation had issued, a few colonies were liberated. During the fall several more colonies were liberated as the stock increased, until over 40,000 Schedius had been colonized.

The Schedius in the reproduction trays continued to increase until early in 1910, when it was estimated that at least 1,000,000 Schedius were on hand. The trays containing this material were placed in a cool cellar until March. In the spring most of the Schedius and parasitized eggs were divided into a few less than 100 colonies and the material was distributed over a considerable part of the moth-infested area of Massachusetts. The Schedius and parasitized eggs not distributed were placed in cold storage for the summer, in an attempt to carry them over so that a stock would be on hand in the fall of 1910 for starting new reproduction trays. No more Schedius were obtained from this material, however, all of which perished before the end of the summer.

DESCRIPTION AND LIFE HISTORY OF SCHEDIUS KUVANAE HOWARD

DESCRIPTION OF ADULT[4]

FEMALE.—Length, 0.99 mm.; expanse, 2.39 mm.; greatest width of fore wing, 0.43 mm. Vertex and cheeks very faintly shagreened; mesoscutum nearly smooth, shining, with minute, rather sparse punctures; mesoscutellum densely and rather coarsely shagreened, well rounded at tip; propleura very faintly shagreened, somewhat shining. General color black; mesoscutellum with a bronzy luster; trochanters, tips of femora, apical half or a little more of front and middle and hind tibiae yellowish; all tarsi lighter; antennae dark brown; dark parts of the legs more brown than black. Wings hyaline. [See fig. 1, *a*.]

MALE.—Length, 0.9 mm.; expanse, 2.28 mm.; greatest width of fore wing, 0.43 mm. Resembles female, except that the flagellum of the antenna is light brown, and except for the structural characters mentioned in the generic diagnosis.

Described from numerous male and female specimens reared September, 1908, at the gipsy moth parasite laboratory of the State of Massachusetts and the Bureau of Entomology, at Melrose Highlands, Mass., from the eggs of *Porthetria dispar* received from Tokyo, Japan, from S. I. Kuwana, Entomologist of the Imperial Agricultural Experiment Station at Nishigahara, Tokyo, after whom the species is named in partial recognition of his great services to the United States in sending parasites from Japan.

TYPE.—No. 12158, United States National Museum; Gipsy Moth Laboratory No. 1698.

OVIPOSITION AND IMMATURE STAGES

Females of Schedius deposit their eggs within the egg of the gipsy moth and if the host larva is developed, as is usually the case, the parasite egg is placed within the body of the caterpillar. The egg (fig. 1, *b*) has a long stalk which is attached to the host egg at the point where the egg of the gipsy moth was punctured by the female parasite. Gipsy-moth eggs which have been exposed to Schedius females in laboratory experiments often have several parasite eggs placed in them by the same or different females. The time required for oviposition varies considerably and records of from 9 to 45 minutes have been obtained. The process usually takes from 10 to 20 minutes.

Oviposition (fig. 2, *a*) often begins during the first day of adult life and extends over several weeks. In one experiment where seven fertilized fe-

[4] HOWARD, L. O. TECHNICAL RESULTS FROM THE GIPSY-MOTH PARASITE LABORATORY. I. THE PARASITES REARED OR SUPPOSED TO HAVE BEEN REARED FROM THE EGGS OF THE GIPSY MOTH. U. S. Dept. Agr., Bur. Ent. Tech. Ser. Bul. 19: 1–12, illus. 1910.

FIG. 1.—*Schedius kuvanae:* *a*, Adult female; *b*, egg; *c*, third-stage larva still retaining egg-stalk and anal shield; *d*, egg-stalk and anal shield of larva as found in host eggs of the gipsy moth from which the adult Schedius has emerged or in which the Schedius larva has been attacked by a secondary parasite; *e*, larval mandibles; *f*, pupa. All greatly enlarged. (Howard and Fiske)

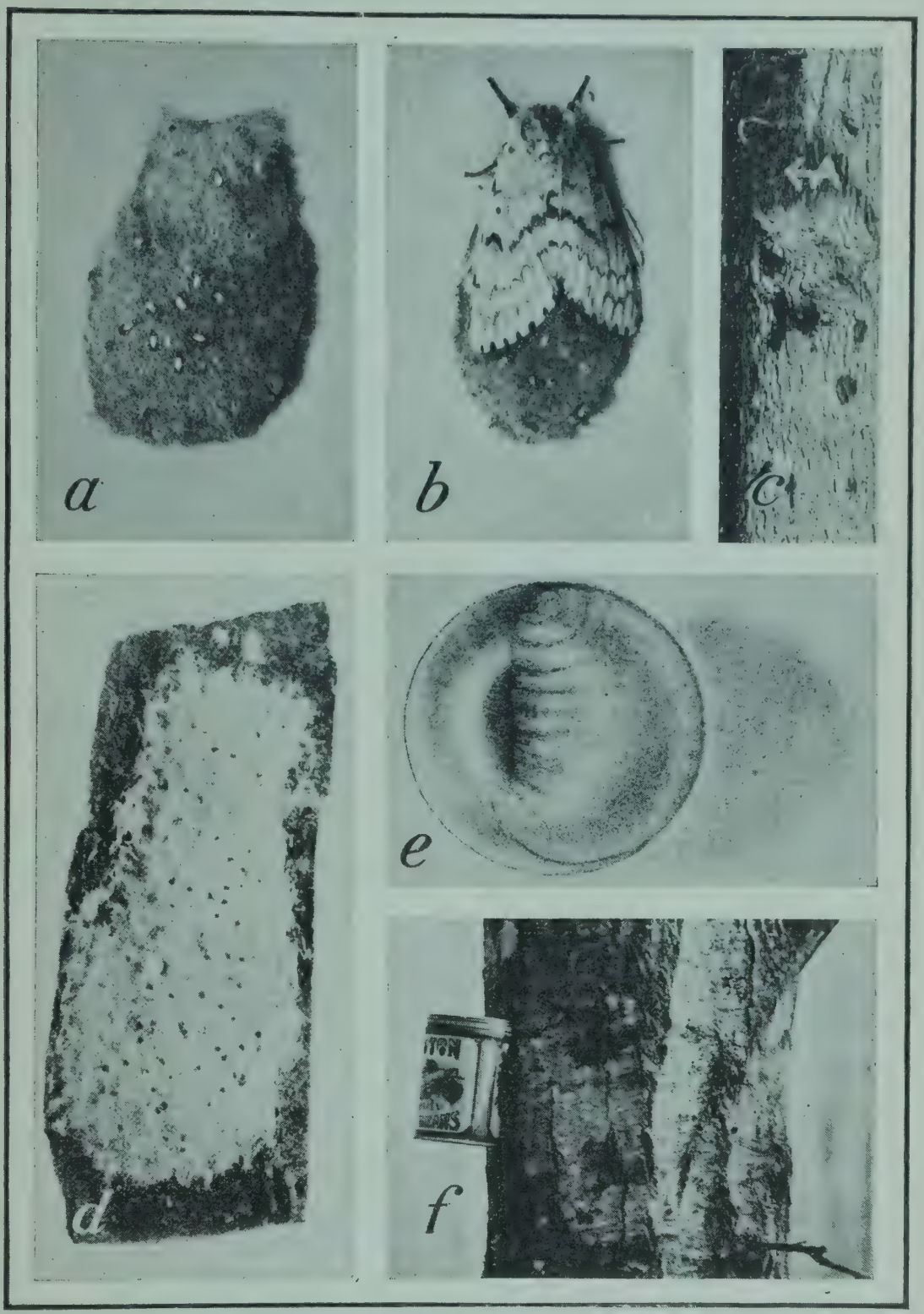

FIG. 2.—*Schedius kuvanae* and *Anastatus bifasciatus: a*, Gipsy-moth egg cluster with adult Schedius on it; *b*, female gipsy moth depositing egg cluster, with female Anastatus on cluster; *c*, Anastatus colonization mark on roadside tree; *d*, gipsy-moth egg cluster showing exit holes of Schedius; *e*, hibernating larva of Anastatus as seen within host egg (Howard and Fiske); *f*, Anastatus colonization can in position on tree trunk

male Schedius were in the presence of gipsy-moth eggs for 23 days, the resulting progeny showed that oviposition had taken place during each one of these days. The females were then transferred to another lot of gipsy-moth eggs and continued to oviposit during the following three weeks. One individual oviposited 27 days later, or 71 days after issuing. The other Schedius oviposited during a period of six weeks. Seventy per cent of the oviposition took place during the first three weeks of adult life.

In regard to the later life of Schedius within the host egg, the following is taken verbatim from Bureau of Entomology Bulletin 91 (p. 180), by L. O. Howard and W. F. Fiske: [5]

> When the egg hatches, the larva does not entirely leave the shell, but remains with its anal end thrust into it, and the stalk, which is hollow, becomes functional and acts like a lifeline attached to a submarine diver in supplying a connection with the outer air. As the larva grows the stalk increases in thickness, and the last anal segment of the larva becomes covered with a thick chitinized shield, which is unaffected by the action of strong caustic potash. There are two larval molts, and consequently three larval stages. During the entire course of both the first and second the young parasite remains quite firmly attached to its anal shield and lifeline and the cast skins are not entirely sloughed off, but are merely pushed backward. After the third ecdysis it retains this connection for a while, and grows rapidly, but about the time when it reaches maturity the connection with the shield is broken, thus proving that it is not part and parcel of the integument. It would appear rather that this shield, including a tube within the egg-stalk (which, as stated, grows in thickness after the egg itself hatches), is actually part of the integument of the first-stage larva, and that the second and third stages merely continue to use what is in effect the skin of the first larval molt.

A third-stage larva, with egg-stalk and anal shield still retained, is shown in Figure 1, *c*, the detached egg-stalk and anal shield at *d*, and larval mandibles at *e*.

The Schedius larva within the gipsy-moth egg consumes the entire embryo if it is in a new egg in which the host larva has not developed. When the Schedius egg is placed within a host egg in which the gipsy-moth larva has developed, the parasite maggot devours the entire caterpillar except the hard chitinized parts and the hair. The hair is left clinging to the inside of the host eggshell. This superficial character is used in determining that Schedius has been present in the egg under consideration.

In 16 to 17 days after the deposition of the parasite egg the Schedius larva is full grown and transforms to its pupa (fig. 1, *f*). The usual period during warm weather from oviposition to the issuance of the adult parasite (fig. 2, *d*) is 21 days. This time varies considerably with the climatic conditions. More time is required for its development during prolonged cool or wet weather and late in the fall. The first spring generation requires about six weeks for development.

FEEDING

In the breeding work at the laboratory adults of Schedius have been fed sugar sprinkled lightly on the inside of banana peeling. A solution of one-half honey and one-half water is also very satisfactory. This solution is placed on paper or blotting paper. (White blotting paper is used, since it was found that Schedius had sometimes died after feeding on this mixture placed on colored blotting paper.) Adult Schedius have been noticed apparently feeding at the punctures made by their ovipositors in gipsy-moth eggs.

LONGEVITY EXPERIMENTS

Schedius adults are quite hardy and live for many weeks when they have food and proper conditions. Records have been obtained of female Schedius living 24 days without food. The male Schedius do not live as long as the females and usually both sexes die within three or four days after issuing if food is not available. The longest records of adult life of Schedius with food are 105 days for males and 130 days for females. Many records have been obtained of Schedius adults living for five and six weeks.

NUMBER OF PROGENY AND PROPORTION OF SEXES

Laboratory experiments to determine the number of progeny from a single female Schedius have shown great variations. Many females die before they have had sufficient time to deposit the full number of eggs of which they are capable. This early mortality of many females under laboratory conditions is probably greater than occurs in the field. In laboratory experiments there probably is more superparasitism of the host eggs than is the case in the field. When more than one Schedius egg is placed in a gipsy-moth egg it is usually wasted, for very rarely does more than one Schedius develop in such an egg.

[5] HOWARD, L. O., and FISKE, W. F. THE IMPORTATION INTO THE UNITED STATES OF THE PARASITES OF THE GIPSY MOTH AND THE BROWN-TAIL MOTH: A REPORT OF PROGRESS, WITH SOME CONSIDERATION OF PREVIOUS AND CONCURRENT EFFORTS OF THIS KIND. U. S. Dept. Agr., Bur. Ent. Bul. 91: 312, illus. 1911.

The largest number of Schedius which has been recorded at the laboratory as developing from a single fertilized female is 191. In these experiments 40 pairs of fertilized Schedius were used. The records of four pairs were 183, 180, 161, and 152. The progeny of several of the other pairs numbered less than 100, and the average progeny from one pair of Schedius, 105. It is probable that under favorable conditions in the field many Schedius deposit at least 200 eggs.

Records obtained at the laboratory show that female Schedius are more abundantly produced from fertilized parents than are males. One typical set of data, concerning over 4,000 adults reared and examined, shows that 74 per cent of the issuing generation were females.

In jars at the laboratory there has been considerable in-and-in breeding. Parents and offspring and brothers and sisters have mated, and the first generation has showed no apparent weakness. In the large reproduction trays (fig. 3, *a*, *b*), in which Schedius are bred for colonization, there is considerable in-and-in breeding. After five or six generations have been produced the resulting Schedius are smaller and weaker than those of the first few generations, probably because of the continued in-and-in breeding.

PARTHENOGENESIS

Schedius reproduces parthenogenetically, the resulting offspring always being males. In laboratory experiments in which females have not been allowed to become fertilized the average number of the progeny has been about 50. This is much lower than the average number of progeny obtained in similar experiments conducted with fertilized Schedius.

SUPERPARASITISM

Gipsy-moth eggs which have been exposed to Schedius in laboratory experiments often contain more than one parasite egg. In these experiments the same female often oviposits more than once within the same host eggs, and occasionally she pierces a host egg with her ovipositor but does not insert an egg. Hundreds of thousands of gipsy-moth eggs collected in the field are examined with a binocular microscope each year, and only a very few cases have been observed where more than one Schedius has developed within an individual gipsy-moth egg.

HIBERNATION

The manner in which this parasite passes the winter was for a long time in doubt. Schedius is present late in the fall in gipsy-moth eggs and will often issue from eggs collected in December. Schedius rarely issue from egg clusters collected after winter begins and temperatures of 15° F. and lower are recorded. Examination of such eggs with a binocular microscope often shows all stages of dead Schedius within the eggs. Hundreds of thousands of gipsy-moth eggs containing all stages of living Schedius have been kept through the winter in cold storage at temperatures around 30° F. Similar lots of eggs have been kept in various types of containers, under as nearly natural conditions as possible, in cages in the laboratory yard. Such experiments have been tried during a number of years but in no case has a single adult Schedius been obtained the following spring.

Many experiments similar to those just mentioned have been conducted with adult Schedius to obtain positive records of adult hibernation. Such experiments often failed to carry Schedius through. In the next succeeding paragraphs are described the first experiments in which the proof was positive that Schedius were carried successfully through the winter as hibernating adults.

A wooden tray (10 by 10 by 4 inches) with a wooden base was prepared for the hibernating experiment. On the inner sides were tacked several small strips of felt and pieces of cotton batting. Several small pieces of such padding were folded and moistened with a solution of one-half water and one-half honey. This tray after being stocked with Schedius was placed in a Riley cage in the yard and left there until completion of the experiment the following summer, except when it was taken into the laboratory for observation.

On October 6, 1915, about 50 adult Schedius and a few gipsy-moth eggs were placed in the tray. The several observations which were made up to November 10 showed Schedius to be active, but many adults had died. On this date a few more adults were added. On November 18 some Schedius were still alive, especially on the sides of the cage close to the felt. On December 21 the temperature at the laboratory yard descended to 13° F., but on December 23 Schedius were still alive. A number were clinging to the sides of the tray

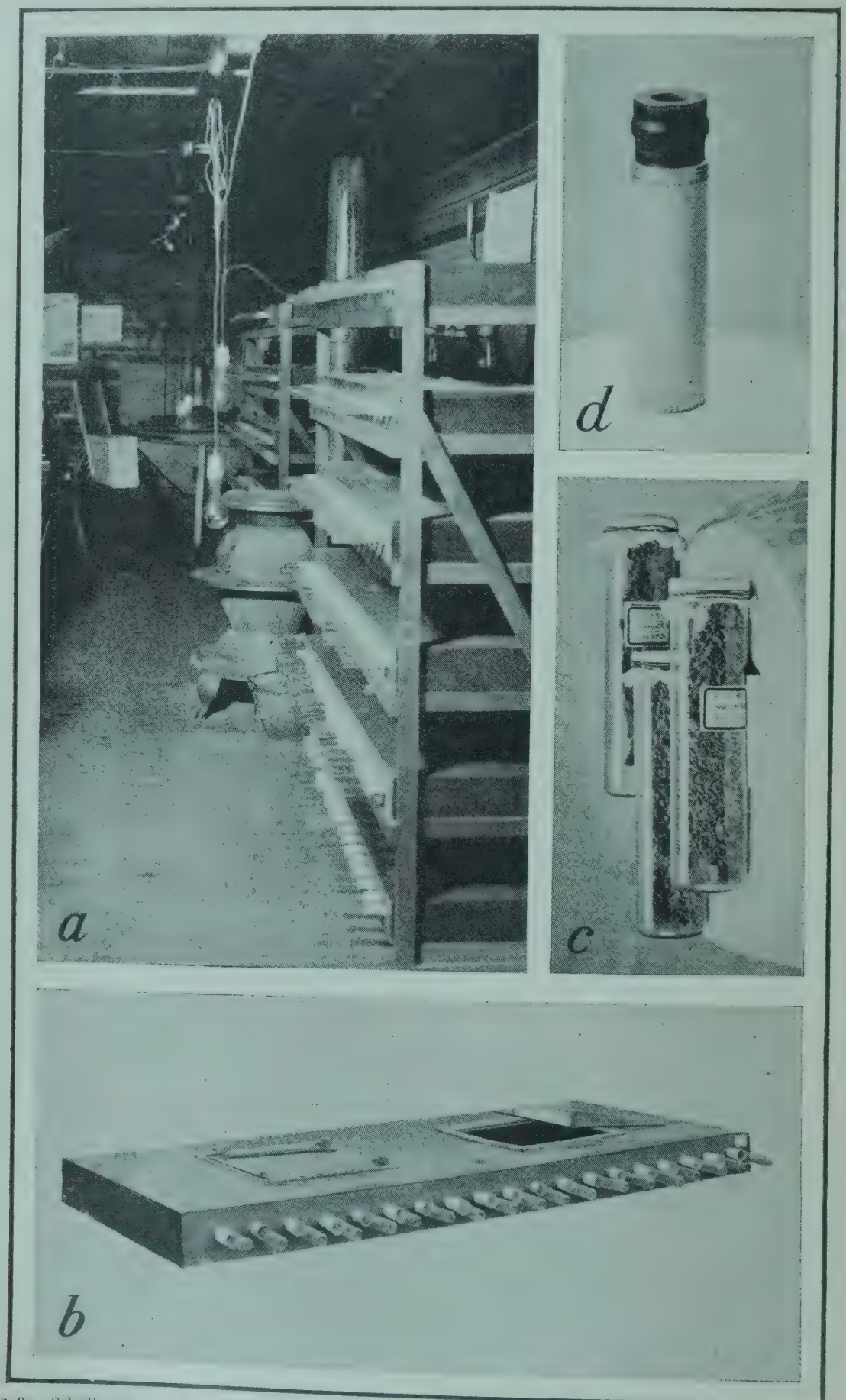

FIG 3.—*Schedius kuvanae* and its colonization: *a*, Two stacks of Schedius rearing trays; *b*, a single rearing tray; *c*, glass vials containing gipsy moth eggs from which Schedius adults were obtained for stocking rearing trays; *d*, mailing tube into which adult Schedius are transferred from the rearing trays. A removable cover in the top of the mailing tube contains a spring trapdoor which prevents the Schedius from escaping. When the tube contains 4,000 Schedius, this cover is removed and the top is covered with a piece of cotton cloth

near the felt and also near the cotton at the base of the cage; some were present in the folded food cloth, and a few were under the gipsy-moth egg masses. On January 3, 1916, all of the gipsy-moth eggs were removed. During the week of January 3 to 10 the thermograph in the yard recorded as low as 6° F. and on several days below 10°. On January 10 the Schedius were observed crowded together in little groups on the walls of the cage near the felt, and a few were in the folds of the food cloth. A few which were brought into the laboratory began to move within 15 minutes. During the week of January 9 to 16 the temperature fell to 3° F. On January 17 a few Schedius were brought into the laboratory; one of these moved in 10 minutes after being in the warm room and several became quite active within 45 minutes. Many which had dropped from the walls of the cage were dead, apparently killed by the cold. The reviving Schedius were in all cases females. There was a small amount of snow on the ground at this time but the Schedius in the cage were probably not so well protected from cold as they would have been if they had been covered with snow.

The temperature in the yard was below zero on February 14 to 16, and on the 14th the thermograph registered −10° F. The ground and cage were covered with over a foot of light snow. The cage was examined on February 16 and many dead Schedius were found on the floor. A few Schedius in the food cloth revived after being exposed for a short time in the laboratory. On March 8 several living Schedius were noted after the tray had been brought into the laboratory for a short time. On March 27, a warm spring day, several Schedius were seen to move in the cage. Again on April 11, a warm sunny day, several Schedius were seen moving about in the cage. A few nonparasitized gipsy-moth eggs were placed in the tray on this date with a small quantity of food (one-half honey and one-half water). On May 8 these eggs were removed, and on May 22 adult Schedius began issuing from them. On April 24 several Schedius were seen active in the cage. On May 2 over 20 adults were running about the cage, and three gipsy-moth egg clusters were placed in the tray. Most of the Schedius were ovipositing in the eggs which had been put in the tray on April 11. After a short time one female oviposited several times in a new egg cluster. On May 13 the second lot of gipsy-moth eggs which were placed in the tray began to hatch. The Schedius were very active and oviposited freely. On May 20 all but six of the hibernated Schedius were dead. The weather had been cool and rainy for several days. On May 27 only two Schedius were alive. On this date two of the three egg clusters which were put into the tray on May 2 were removed. On June 25 two Schedius issued from the two egg clusters removed. One hibernated Schedius was alive on June 2, and one female Schedius was still alive on June 24. When the tray was next observed a generation of Schedius had begun to issue from the single egg cluster which remained in the cage, so that it was impossible to know how long after June 24 the hibernating female had lived. The mortality of Schedius late in the fall is enormous and only a very few strong females in especially favorable hibernating quarters survive. It is probable that the females which hibernate successfully are fertilized in the fall but do not oviposit, for the first individuals found early in the spring are always large females with greatly extended abdomens. Both sexes are present in the progeny developed from these hibernating females. It is probable that certain individuals enter hibernating quarters at various times during the fall and thus lessen the chance of extermination should a sudden drop in temperature late in the fall kill all of the active individuals.

NUMBER OF GENERATIONS

The records obtained from hibernating experiments carried on at the laboratory have been substantiated by field observations. Schedius which have hibernated successfully become active on the first warm days of April and are occasionally observed ovipositing during the latter part of this month in the overwintering gipsy-moth eggs. They are scarce at this early date and it is probable that the first few seen in April have hibernated in protected spots which were warmed by the early spring sun. Only rarely do Schedius issue from eggs collected before the middle of April, and the majority do not leave their winter quarters until the last week of this month. These adults, which are always females (at least no males have been found in the spring until there has been ample time for a spring generation to develop), live three to five weeks, so that the oviposition period is prolonged over several weeks. Six weeks are required for the first spring generation to develop on the overwin-

tering gipsy-moth eggs. It follows that adults of the first spring generation begin issuing from gipsy-moth eggs about the last week of May and continue well into June, the greater number issuing during the first half of June. Both sexes are present in this and future generations, indicating that some, if not all, of the females which hibernated successfully were fertilized during the preceding fall. Many of the adults of the first spring generation oviposit in nonhatched gipsy-moth eggs. Adults of the second spring generation are found issuing from the beginning to the middle of July. Experiments have shown that adult Schedius often live five to six weeks, ample time being thus afforded for many adults of the first generation to oviposit in the new gipsy-moth eggs which are laid in July.

Adult Schedius are often seen ovipositing in gipsy-moth eggs before the entire cluster is deposited by the moth. The first fall generation issues about 21 days after oviposition, or during the first week in August. A part of the first fall generation often has issued before all of the gipsy-moth eggs have been deposited. The generations during the fall so overlap one another that they can not be definitely separated, but there is time during the average season from the middle of July until early in November for four complete generations and a partial fifth. During the warm weather from the middle of July to the middle of September there is a generation about every 21 days. The last two generations require somewhat more time in which to develop and many of the Schedius within the host eggs which would otherwise become adults of the fifth generation are killed in various stages of development by severe temperatures.

The approximate time of the appearance of each generation of Schedius is here given. The dates vary considerably during different seasons.

April 10 to May 15: Hibernated female Schedius active in the field.

May 25 to June 8: Adults of the first spring generation, both sexes, issuing from host eggs.

June 30 to July 15: Adults of the second spring generation (partial) issuing from nonhatch gipsy-moth eggs.

Week of August 5: Adults of the first fall generation issuing.

Week of August 26: Adults of the second fall generation issuing.

Week of September 16: Adults of the third fall generation issuing.

Week of October 14: Adults of the fourth fall generation issuing.

November 11 and later: Adults of the fifth fall generation (partial) issuing. Cold weather causes the death of many of this generation within the host egg.

REPRODUCTION ON GIPSY-MOTH EGGS

Schedius reproduces on freshly deposited eggs of the gipsy moth as well as on those in which the embryo is fully developed. Freshly deposited eggs have been kept for a month in cold storage at a temperature of about 30° F. and then exposed to Schedius. An examination of these eggs showed no development of the embryo, and Schedius reproduced readily on such eggs. During the major part of the breeding period of the Schedius the host eggs are well developed, and at such time Schedius acts as a parasite of the unhatched gipsy-moth larva within the host egg.

Many records have been made of the breeding of Schedius on dead gipsy-moth eggs. Some of the early work on breeding at the laboratory was greatly facilitated by using gipsy-moth eggs which had purposely been killed by being placed in hot water. In this manner the breeding was continued during the winter and spring months without interference from the hatching of gipsy-moth larvae. Gipsy-moth eggs have been held in cold storage for two years until all or practically all of them were dead, and then have been used successfully for reproducing Schedius. Some of the adult Schedius from the first spring generation oviposit successfully in gipsy-moth eggs which have failed to hatch earlier in the spring. Schedius bred from dead gipsy-moth eggs are not so strong and robust as when bred on healthy gipsy-moth eggs.

REPRODUCTION ON EGGS OTHER THAN THOSE OF THE GIPSY MOTH

Schedius has been bred successfully at the laboratory in eggs of the following: *Hemerocampa leucostigma* S. & A., *H. definita* Pack., *Callosamia promethea* Dru., *Malacosoma americana* Fab., *Hemileuca maia* Dru., *H. oliviae* Ckll., *Euproctis chrysorrhoea* L., and *Stilpnotia salicis* L. It is interesting to note that in a few cases of parasitism of a large egg like that of *H. oliviae*, several Schedius developed in an individual egg. On two occasions five, and on a number of occasions two and three, Schedius issued from a single egg. Parasitism of these species by Schedius in the field is probably of rare occurrence, and in no case have Schedius been reared from any of the eggs above

named which have been collected in the field. The fact that it is possible for Schedius to reproduce on such a variety of host eggs is important, and it may yet be found parasitizing these and other lepidopterous eggs when conditions are favorable.

SCHEDIUS AS A HYPERPARASITE ON APANTELES MELANOSCELUS RATZ

One of the parasites of the gipsy moth which have been imported from Europe is *Apanteles melanoscelus.* Among 87,000 cocoons of this species which have been collected during the last three years to be used in colonization work, 11 were found from which *Schedius kuvanae* had issued. These cocoons had been isolated in gelatin capsules, in which the Schedius were found. As a hyperparasite on *A. melanoscelus,* it often happens that more than one Schedius issues from a single cocoon; on one occasion 17 adult Schedius so issued.

DEVELOPMENT OF SCHEDIUS ON LARVAE OF ANASTATUS

Schedius developed on full-grown larvae of *Anastatus bifasciatus* in some of the gipsy-moth eggs introduced from Japan. Many attempts have been made at the laboratory to rear Schedius on gipsy-moth eggs which have been parasitized by Anastatus. It is surprising that in only one case has this resulted in producing a generation of Schedius. Many hundreds of thousands of field-collected gipsy-moth eggs have been examined with a binocular microscope in the course of the work during the last 14 years, but there is no record of Schedius issuing from an egg which previously had contained Anastatus. In a few instances Schedius and Anastatus larvae have been found within the same egg, but in such cases neither has issued.

These two parasites are not often found to be abundant in the same locality. The examination of eggs of the gipsy moth usually shows one or the other in a great majority. In a few places both species are occasionally about equally represented. It seems probable that in some areas these parasites must conflict, but it is not apparent which one is the survivor. From the habits of the two species it would seem that Anastatus would suffer in an area where Schedius is abundant. In laboratory experiments Schedius has been observed to oviposit in gipsy-moth eggs containing Anastatus, but these eggs have not developed except in very rare cases, and in those the Schedius have not issued.

LATER REPRODUCTION WORK AND APPARATUS USED TO OBTAIN SCHEDIUS KUVANAE FOR COLONIZATION

Many types of containers have been tried in the development of the present apparatus used for breeding Schedius. In the first work on reproduction, when very few Schedius were available, small glass vials (1 by 4 inches) containing a few gipsy-moth eggs were used. Later, as the Schedius became more abundant, a larger glass vial (8 by 2 inches; fig. 3, *c*) was found more satisfactory. With the increase of the parasites wooden trays (14 by 14 by 2½ inches) with glass tops and bottoms of cotton cloth were found to serve the purpose better.

In August of each year a few gipsy-moth eggs were collected at locations where the parasite was present and placed in glass tubes (8 by 2 inches) for the issuance of Schedius. The tubes were examined often during the day and all of the adult Schedius were removed and transferred to the breeding trays, in which had previously been placed a supply of masses of gipsy-moth eggs. As the breeding work increased larger trays (5 feet long, 2½ feet wide, 3 inches deep) were used. These were lined with black paper and covered with white cotton cloth, but were not satisfactory, for if the paper broke, as it often did, many adult Schedius, trying to get to the light, were caught between the paper and the cloth and died there. A very satisfactory tray has at last been developed (fig. 3, *b*), made of matched boards of half-inch cypress with corners dovetailed so as to be tight. The bottom and top are of the same material. The tray, by outside measurement, is 5 feet long, 2 feet wide, and 5 inches deep, and is painted on the inside with flat black paint. Two holes, each 12 inches square, are cut in the top, each halfway between the center and end of the tray, so that the eggs can be properly spread over the bottom and light may enter. The holes are covered with window glass. In the front of the tray are 19 circular holes, 1 inch in diameter, usually kept closed with cork stoppers.

During the latter part of August or early in September the bottoms of these trays are covered evenly with a half-inch layer of gipsy-moth eggs. The holes in the front of the trays are plugged with cork stoppers until it is necessary to draw out the Schedius. Then a few gipsy-moth eggs collected from places where Schedius are plentiful are placed in each tray. The trays

are stocked at intervals of several days, so timed that after the resulting generations begin to issue the flow will be gradual and continuous; for it would be difficult to handle so many adult Schedius should they all appear at the same time. After the trays are stocked they continue to yield Schedius for four or five generations without further care except to feed the adults and to keep the room warm. In from 21 to 25 days after the introduction of Schedius into the breeding trays a new generation of parasites issues.

In order to draw the adult Schedius from the breeding trays it is necessary to exclude the light, which is done by placing pieces of black paper over the glass in the tops. The cork stoppers are removed from the front of the trays and glass vials (4 inches by 1 inch) are substituted. The vials are held in place by means of small paper cones inserted in each hole. Electric lights are hung in front of the trays (fig. 3, *a*). As the Schedius enter the vials each vial is replaced by an empty one and an estimate is made of the Schedius in the vial withdrawn. The adult Schedius are then transferred from the vial into a mailing tube 8 by 3 inches in size. To do this, in the top of the mailing tube is placed a removable cover with a trapdoor through which the open end of the glass vial containing the Schedius is inserted (fig. 3, *d*). By tapping the bottom of the inverted vial the Schedius drop into the mailing tube. When the glass vial is withdrawn the door springs back into place, preventing the Schedius, which are very active, from escaping. When the tube contains about 4,000 Schedius a slip of paper smeared with a solution of one-half honey and one-half water is placed in it. The mailing tube is then covered with a piece of cotton cloth held in place by an elastic band. The colony is now ready for liberation and each day's output is sent by mail or otherwise to men in the field.

COLONIZATION OF SCHEDIUS

The first colonization of Schedius in New England was made in the fall of 1909. Colonies were liberated in five different towns. About 1,000 adults which issued from Japanese eggs were colonized. The others liberated were bred through a series of generations at the laboratory. In the spring of 1910 large numbers were liberated in 89 different towns. This colonization material was obtained from the stock used in the fall of 1909. Each year since 1910 Schedius has been colonized farther out from Melrose, Mass., with stock obtained in New England, until at present over 20,500,000 Schedius have been liberated and 358 towns in New England have been colonized. Three towns in Maine have been colonized, 95 in New Hampshire, 203 in Massachusetts, 31 in Rhode Island, and 26 in Connecticut. New Jersey has received nearly half a million Schedius, Washington, D. C., about 20,000, and Illinois 17,000. The Schedius were sent to the last two places in order to test their ability to attack the eggs of the tussock moth, which were abundant at the time of liberation. A few Schedius have been sent to Maxwell, N. Mex., in connection with the investigation of the range caterpillar (*Hemileuca oliviae*). About 200,000 specimens were sent to Madrid, Spain, in the fall of 1923, where they are being bred for liberation in a heavy gipsy-moth infestation in the Royal Forest near Madrid. *Schedius kuvanae* is not present in Europe, and this parasite, if successfully introduced, should develop into an important enemy of the gipsy moth in Spain. It is likely to prove more effective in Spain than in New England, because the summer season in Madrid is longer, allowing for more generations of Schedius to develop and the winters are less severe than in the United States.

The area which has been colonized in New England is shown in Figure 4.

Table I gives the colonization records in a much abbreviated form.

The colonization of Schedius is carried on during the fall, beginning about the middle of September and continuing into November, until the weather gets too cold for insect activity. As the colonies are prepared at the laboratory for liberation they are sent to men in the field for liberation or taken directly to the field for colonization. The colonies are placed from 1 to 2 miles apart where woodland and gipsy-moth infestations warrant it. Each tube contains 4,000 Schedius for liberation in suitable places in the area to be colonized. If the day is warm the Schedius are active and it is only necessary to remove the cloth top from the tube, when a few gentle taps will empty the tube of the parasites. The men liberating Schedius are supplied with a blue-print map, similar to that shown in Figure 11, indicating the roads in the town. The exact location of each colony is recorded on the map. In addition, a note is written giving the details of the colony site, so that the place may be visited at any later occasion. A roadside tree is marked with white paint with the letter "*S*" and an arrow pointing to the colony.

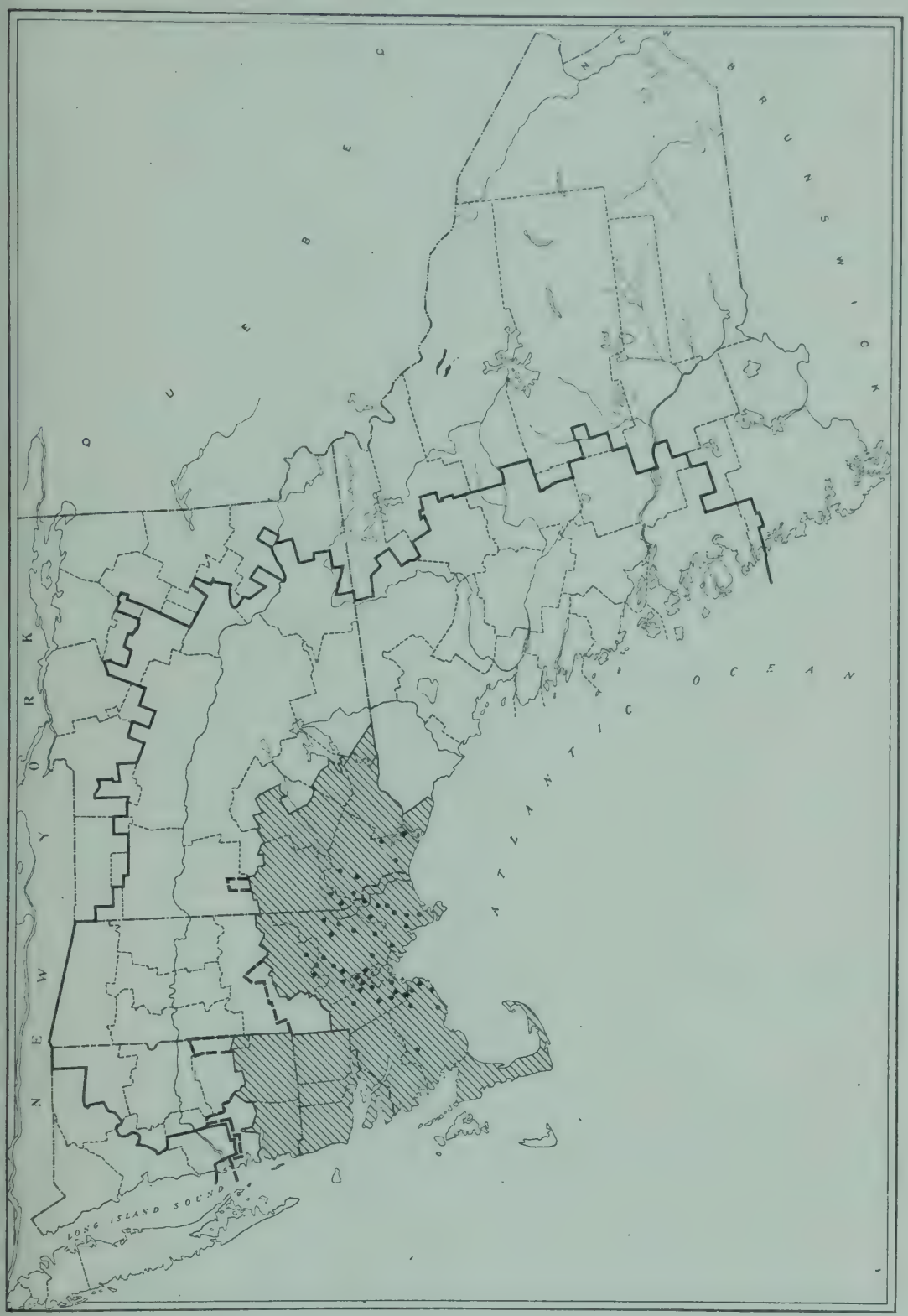

FIG. 4.—Map showing colonization of *Schedius kuvanae* in New England. The heavy black line is the quarantine line for the gipsy moth (*Porthetria dispar*), the area to the right of this line being infested by that insect. The shaded area has been solidly colonized with Schedius. The area between the broken line and the shaded area has been partially colonized with Schedius. The dots show the locations of the observation points from which egg collections have been made

TABLE I.—*Summary of the colonization of Schedius kuvanae*

Year	Massachusetts		New Hampshire		Rhode Island		Maine		Illinois	
	Colonies	Individuals	Colonies	Individuals	Colonies	Individuals	Colonies	Individuals	Colonies	Individuals
1909	6	46,415								
1910	89	889,120								
1911	9	68,486								
1912	7	93,368	2	19,807						
1913	111	382,645								
1914	247	1,120,536	252	944,725	2	21,855				
1915	108	455,146	52	182,308						
1916	234	1,477,963	265	1,161,929	15	57,200				
1917 [a]	3	9,120	343	1,006,279			77	232,260	3	5,000
1918	945	3,844,500	237	932,000						
1919			368	1,540,000						
1920					110	440,000				
1921	4	496,000			9	124,000			1	12,000
1922	26	200,000	16	516,000						
1923					118	472,000				
Total	1,789	9,083,299	1,535	6,303,048	254	1,115,055	77	232,260	4	17,000

Year	New Jersey		Connecticut		District of Columbia		Spain		Total	
	Colonies	Individuals	Colonies	Individuals	Colonies	Individuals	Colonies	Individuals	Colonies	Individuals
1909									6	46,41
1910									89	889,12
1911									9	68,48
1912									9	113,17
1913									111	382,64
1914									501	2,087,11
1915									160	637,45
1916									514	2,697,09
1917 [a]									426	1,252,65
1918									1,182	4,776,50
1919									368	1,540,00
1920	67	270,000							177	710,00
1921			219	1,638,875	1	20,000			234	2,290,87
1922	53	212,000	223	904,000					318	1,832,00
1923			201	804,000				[b] 200,000	319	1,476,00
Total	120	482,000	643	3,346,875	1	20,000		200,000	4,423	20,799,53

[a] A few eggs of *Hemileuca oliviae* parasitized by *Schedius kuvanae* were sent to Maxwell, N. Mex.
[b] Estimated colonies included in totals of colonies.

SUCCESS OF COLONIES AND DISTRIBUTION

Records obtained at the laboratory show that about 50 per cent of the colonies of Schedius liberated in Massachusetts and Rhode Island have become established. It is probable that in reality a higher percentage of the colonies are successful. Many of the collections of host eggs from which this percentage was obtained were made one or two years after the colony was liberated. This species is rather slow in increasing to sufficient numbers after its liberation to make it easily recovered. Schedius have been recovered from a total of 150 towns in New England. These recoveries have been made from 125 towns in Massachusetts, 15 in New Hampshire, 9 in Rhode Island, and 1 in Connecticut. The recoveries from the colonies in Maine and New Hampshire have been very poor, except in a few towns in southern New Hampshire. The broken black line in Figure 5 indicates the present dispersion of Schedius in New England. There have been occasional though infrequent recoveries of Schedius north of this line in Maine and New Hampshire. The recovery of Schedius from only one town in Connecticut is not a sign that the species will not do well in that State. No colonies were liberated there until the fall of 1921, and because of the scarcity of gipsy-moth eggs very few have been examined in an attempt to make recoveries.

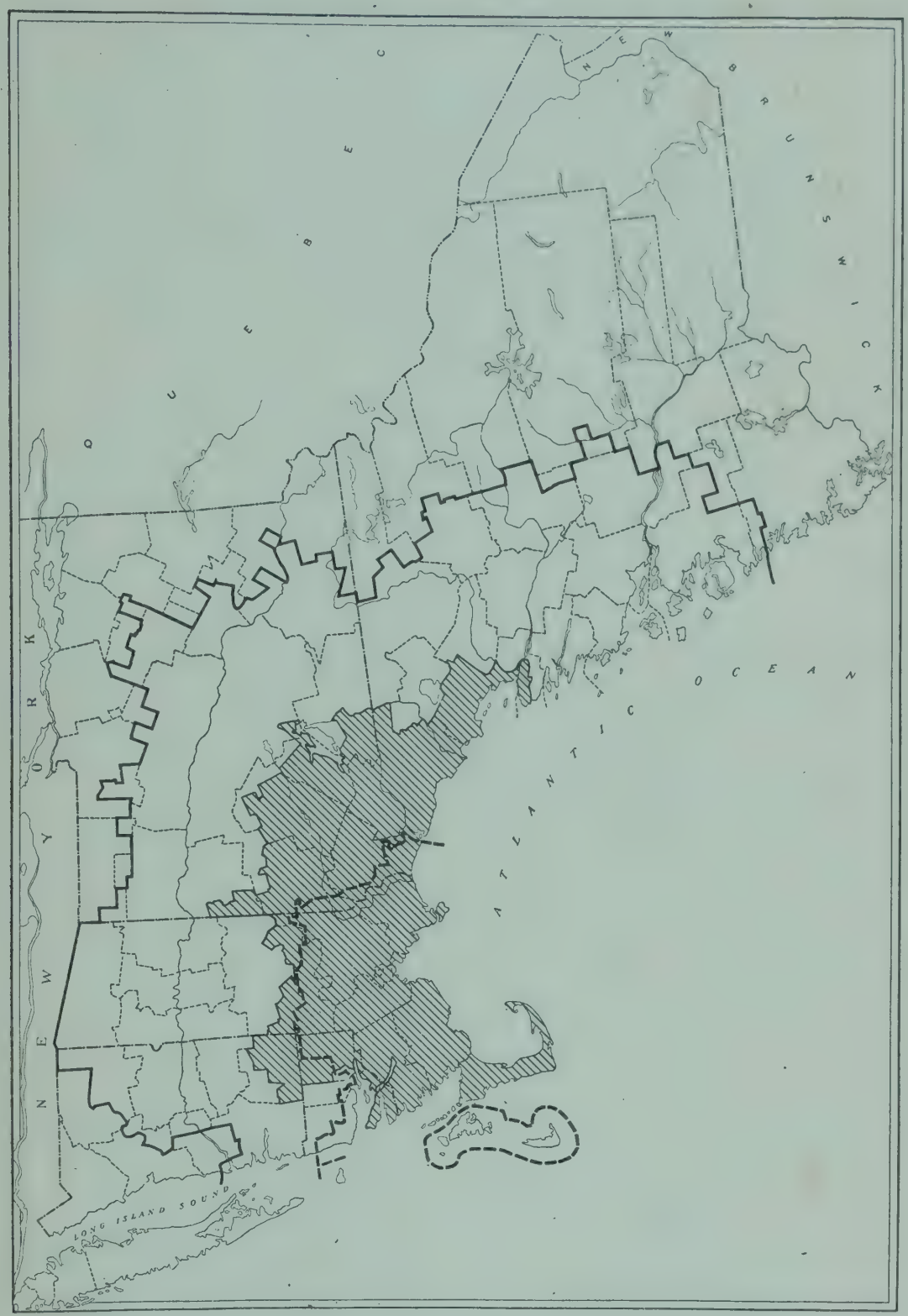

FIG. 5.—Map showing areas in New England from which *Schedius kuvanae* and *Anastatus bifasciatus* have been recovered. The outside heavy black line is the quarantine line for the gipsy-moth (*Porthetria dispar*), the area to the right of this line being infested by that insect. Anastatus has been recovered from the shaded area. Schedius has been recovered from the area inclosed within the broken black line

DISPERSION

Studies of dispersion of Schedius have continued over several years. The method used was to take the point of liberation of a colony as a center and run out lines from this center in the four cardinal directions. Along these lines at points equally distant from the center and consecutively from each other, collections of 10 or more gipsy-moth egg clusters were made. A study of the notes made from the examination of these eggs gave records of dispersion of 300 to 400 yards for the adults of a single generation, or a seasonal spread of over one-half mile. Dispersion records obtained late during the second fall after colonization have shown a spread of nearly 2 miles for the two seasons. Female Schedius are able to fly, and the wind may carry the adults considerable distances.

EARLY METHOD OF OBTAINING ANASTATUS BIFASCIATUS FROM IMPORTED GIPSY-MOTH EGGS FOR COLONIZATION

Anastatus adults were first reared at Melrose in the spring of 1908 from gipsy-moth eggs which were received from Japan and Russia during the winter of 1907 and 1908. This material was kept in a warm room, which hastened the issuance of the parasites. As the Anastatus began to emerge, they were placed with gipsy-moth eggs which had been kept in cold storage since the previous fall in an endeavor to retard the larval development. No reproduction occurred from this experiment, and the imported eggs containing the Anastatus were placed in cold storage to retard the issuance of the parasites until new gipsy-moth eggs were available. In July the imported material was removed from cold storage and 513 adult Anastatus issued and were liberated. During the fall of 1908 and the spring of 1909 large shipments of gipsy-moth egg masses were received from several European countries and from Japan. The largest quantity of the European material came from Hungary. The nonparasitized eggs were allowed to hatch and the remaining parasitized eggs were divided into equal lots for colonization. About 5,000 parasitized eggs were separated from the Japanese eggs and were added to the European colonization material.

DESCRIPTION AND LIFE HISTORY OF ANASTATUS BIFASCIATUS FONSCOLOMBE

Anastatus bifasciatus is now quite abundant in New England, and is often reared from eggs of the gipsy moth. It has previously been known as *Cynips bifasciata* Fonscolombe, *Eupelmus bifasciatus* Foerster, and *Eupelmus bifasciatus* Wachtl.

The early descriptions of this species are rather brief, and are obtainable in only a few libraries. A description, therefore, seems desirable at this time.

REDESCRIPTION (BY C. F. MUESEBECK, OF THE GIPSY MOTH LABORATORY)

FEMALE (fig. 6, *a*).—(Length 2.2 to 3 mm. Head transverse, more than twice as broad as thick anteroposteriorly, the eyes strongly divergent below; vertex finely transversely sculptured posteriorly, minutely punctato-reticulate anteriorly; frons mostly smooth and shining along the eye margins, scrobes strongly transversely sculptured; malar space nearly half as long as the eye, mostly punctato-rugulose, sometimes weakly so; malar groove distinct; ocellocular line about equal to the diameter of an ocellus; antennae inserted on a line with the lower extremities of the eyes or very slightly below; scape extending to the vertex; pedicel at least two-thirds as long as the first funicular segment; funicular segments becoming gradually shorter and broader, the sixth and seventh not distinctly as long as broad; club three-segmented, very nearly as long as the three last funicular segments combined; median lobe of mesoscutum completely strongly punctate, the lateral lobes very faintly lineolated or reticulate, and strongly shining; scutellum and axillae sculptured exactly like median lobe of mesoscutum; mesopleura finely lineolated, shining; propodeum polished; abdomen a little shorter than the thorax, broadening gradually from base to apex where it is broadly rounded, perfectly smooth at base, finely transversely lineolated posteriorly; ovipositor sheaths protruding very slightly beyond apex of abdomen. Head green, with purplish reflections; scape yellow; pedicel and flagellum bronzy-black; mesoscutum dark greenish bronze on the lateral lobes, purplish blue behind the median lobe which is a brilliant golden bronze; scutellum and axillae like the median lobe of mesoscutum except that they are tinged with green; propodeum purplish laterally; mesopleura brownish yellow, darker anteriorly; legs dark brown; wings hyaline with a small faintly fuscous spot at extreme base, and with two broad fuscous bands separated by a complete transverse curved hyaline band which arises directly before the stigmal vein; apex of wing hyaline; abdomen mostly greenish black with a large yellowish spot on first tergite.

MALE.—Length 1.8 to 2 mm. Head transverse, much more than twice as broad as thick anteroposteriorly; eyes very faintly and sparsely hairy; ocellocular line hardly as long as diameter of an ocellus; vertex and frons closely shallowly punctate; lower part of face confluently punctate, weakly so toward the malar furrows; cheeks faintly longitudinally sculptured; malar space more than half the eye height; antennae inserted about on a level with the lower eye margins; scape short, broad, slightly concave outwardly, and scarcely attaining vertex; pedicel very short, about one-third the first funicular segment; flagellum long, of uniform thickness; first segment of funicle twice as long as thick, longer than the second; second and third subequal, fourth, fifth, sixth, and seventh

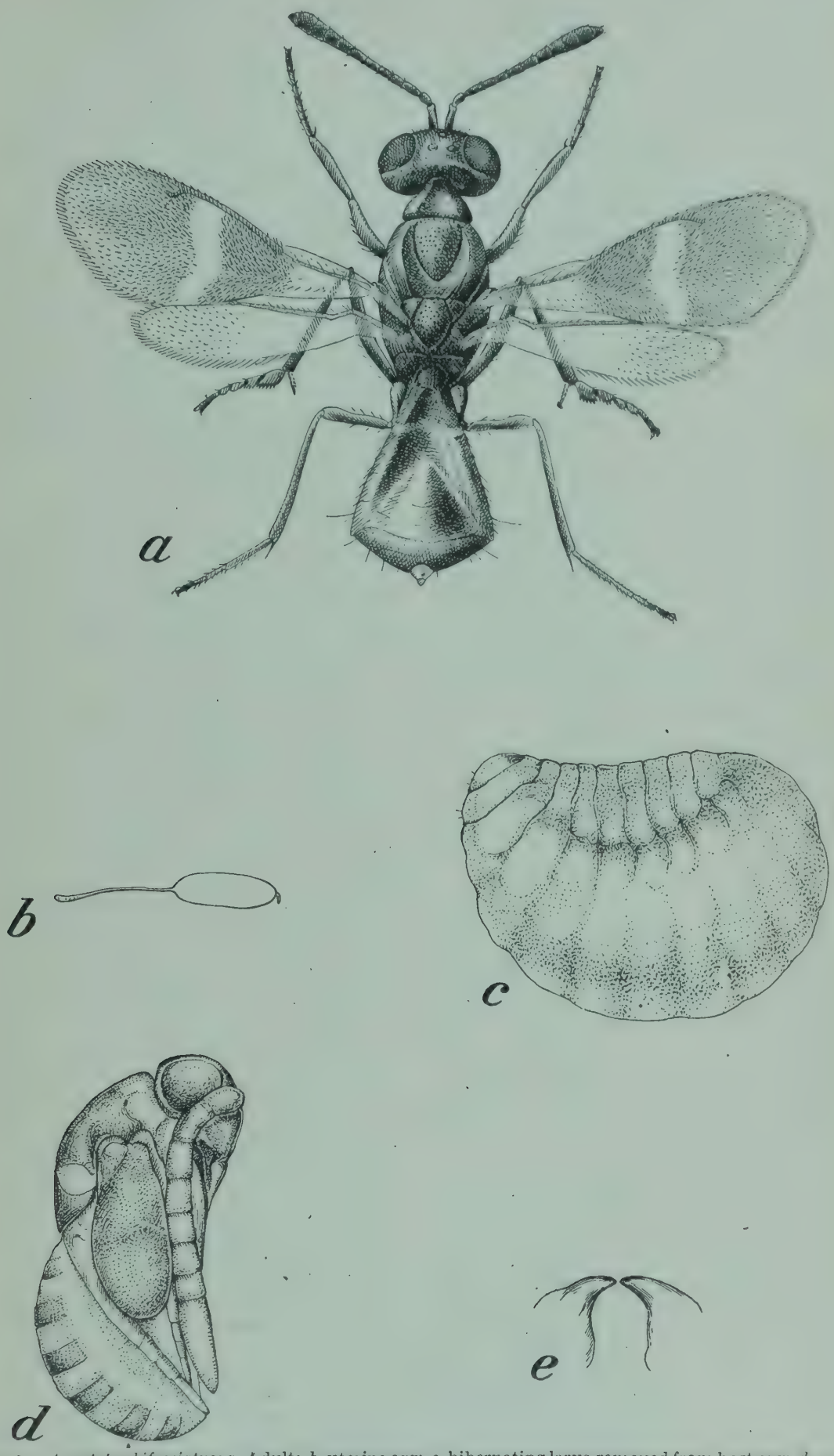

FIG. 6.—*Anastatus bifasciatus: a*, Adult; *b*, uterine egg; *c*, hibernating larva removed from host egg; *d*, pupa; *e*, mandibles. All greatly enlarged (Howard and Fiske)

gradually shorter, the seventh as broad as long; club not distinctly segmented, as long as the three last segments of funicle combined; mesoscutum, axillae, and scutellum rather evenly and minutely reticulato-punctate; propodeum very faintly reticulate, strongly shining, with a median longitudinal carina; spur of middle tibia longer than first tarsal segment; abdomen shorter than thorax, narrowest at base, smooth and shining, with a very faint suggestion of sculpture posteriorly. Head greenish, more or less aeneous black on vertex; antennae black, scape yellow below; thorax greenish black, scutellum bronzy black; pleura with bluish or purplish reflections; propodeum green; wings hyaline; all coxae and femora, base of anterior and middle trochanters and the posterior femora, except at base, black; posterior trochanters, all tibiae, and tarsi pale yellow; abdomen bluish black.

Described from many specimens reared from *Porthetria dispar* Linnaeus at the gipsy-moth laboratory.

HABITS OF ADULTS AND ABUNDANCE IN THE FIELD

Investigations which have been made to determine whether the female Anastatus can fly have indicated that they can not. They jump well and are probably spread short distances by the wind. The males, which issue from hibernation a few days before the females, are often seen flying about the colonization cans in which they were colonized, apparently waiting for the issuance of the females.

Female Anastatus have been observed apparently feeding at the punctures they have made in gipsy-moth eggs, after oviposition or apparent oviposition. In laboratory experiments a mixture of about 40 per cent honey and 60 per cent water has been found the best food tried.

During the last two weeks of July and the first week of August Anastatus adults are found quite abundantly on the gipsy-moth eggs, as in *b* of Figure 2, in most of the area within which this parasite has been generally colonized. In a few locations where the parasite is especially abundant practically every egg cluster will have from 1 to 7 or 8 female Anastatus working on it, while many egg clusters have been observed with 10 to 15. As high as 22 female Anastatus have been seen on a single egg cluster.

OVIPOSITION AND IMMATURE STAGES

As soon as females of the gipsy moth begin their deposition of eggs in July, adults of Anastatus are present and occasionally are found on the abdomen of the female moth even before she begins to lay her eggs. When adults of Anastatus are abundant they begin parasitizing the gipsy-moth eggs almost as soon as they are laid and often five and six Anastatus are observed ovipositing in the eggs before the cluster is entirely deposited (fig. 2, *b*).

The Anastatus female requires less time for oviposition than does the Schedius. The time occupied by Anastatus females in ovipositing varies from 2 to 15 minutes, each oviposition averaging 4 or 5 minutes. S. M. Dohanian, an assistant at the laboratory, while making field observations on Anastatus, saw a single female apparently oviposit 42 times in 2 hours and 15 minutes.

The uterine egg, a hibernating larva, and a pupa of Anastatus are shown in Figure 6, *b*, *c*, *d*, and the larval mandibles in Figure 6, *e*. The parasite egg hatches very soon after oviposition and the resulting parasite maggot develops quickly into the hibernating stage (fig. 6, *c*; fig. 2, *e*), about three weeks being required for the entire development from egg to full-grown larva.

NUMBER OF GENERATIONS

Normally, Anastatus has only a single generation. The adults issue from the gipsy-moth eggs over a considerable period. A few begin to appear during the middle of June, and egg clusters collected as late as August 8, laid the previous year, have given adult Anastatus.

Issuance of Anastatus adults in June and in the middle of August are extremes. The main issuance of adults occurs during July, with the bulk of the Anastatus appearing during the last two weeks of that month. The dates vary with the season, but the heavy issuance occurs at about the time when the majority of the female gipsy moths are depositing egg clusters. The time spent by Anastatus within its host is usually about a year, although this period ranges in length from 10 to 13 months.

In some seasons a few Anastatus have two generations. Usually this is rare, but during the fall of 1921 it was commonly observed. Many egg collections received during the fall gave 2 and 3 per cent of fall issuance of male and female Anastatus. In several experiments these were mated and the females oviposited readily in gipsy-moth eggs. These parasitized eggs were kept through the winter, and during the following summer adult Anastatus issued. In most cases they were males, but there is at least one record of both sexes maturing in the second generation.

The experiment here described was so unusual in its results that it is worth recording. On July 9, 1921, a small breeding tray was stocked with 1,278 new gipsy-moth eggs. Honey-and-

water solution on blotting paper was put in the tray and 150 adult Anastatus which had issued from hibernation at about this time were introduced. The female Anastatus began to oviposit immediately. The gipsy-moth eggs were examined on August 9 and many Anastatus pupae were seen. From August 10 to 20, 172 male and 28 female Anastatus issued. A few days later 18 more adults appeared; the sex was not noted. An examination of the eggs showed that 50.3 per cent of them had been parasitized by Anastatus, and of this 50.3 per cent, 17 per cent had issued and 33.3 per cent were typical hibernating Anastatus larvae. The hibernating larvae were kept through the winter, and the following summer 75.9 per cent of them issued.

The adult Anastatus from this experiment, together with others which issued during the fall from field-collected gipsy-moth eggs, were used in reproduction experiments. These females of the fall generation were observed to mate and to oviposit. The eggs in which they deposited were saved through the winter and adult Anastatus issued from many of them in the following summer.

LONGEVITY EXPERIMENTS

In laboratory trays adult Anastatus are short-lived as compared with the adults of Schedius. They usually do not live much more than 10 days to 2 weeks. Occasionally adults have lived in confinement for 3 weeks.

Cold storage experiments have shown that the Anastatus larvae can live over a long period. Gipsy-moth eggs containing Anastatus hibernating larvae were collected in the spring and placed in cold storage at a temperature of about 30° F. There they were kept until a year from the following July, when they were brought to the laboratory. In August both sexes of adults issued, the Anastatus larvae having lived two years within the host egg. These females oviposited successfully in new gipsy-moth eggs, and the following summer their progeny issued.

REPRODUCTION ON GIPSY-MOTH EGGS

Normally, Anastatus oviposits in fresh gipsy-moth eggs and its larva develops quickly, consuming the entire egg contents before the gipsy-moth embryo develops. Occasionally it is found ovipositing in the field in gipsy-moth eggs which have developed. Laboratory reproduction experiments have been successful, using gipsy-moth eggs in which the embryo was fully developed. A generation of Anastatus has also been developed on dead gipsy-moth eggs which had been placed in cold storage before the gipsy-moth embryo had developed.

REPRODUCTION ON EGGS OTHER THAN THOSE OF THE GIPSY MOTH

Anastatus females attack the eggs of the gipsy-moth more freely than they do the eggs of other insects to which they have been given access in laboratory cages. Females have been confined in cages with various lepidopterous eggs, but oviposition, with reproduction, has not been observed in these experiments except on the eggs of *Hemileuca oliviae* and *Hemerocampa leucostigma*.

REPRODUCTION ON APANTELES MELANOSCELUS RATZ.

During the years 1921, 1922, and 1923 over 87,000 cocoons of *Apanteles melanoscelus* were collected from the field in New England for reproduction and rearing. It was necessary to isolate each of these cocoons because of the enormous amount of hyperparasitism. From this great mass of material four adults of *Anastatus bifasciatus* have been reared.

WINTER MORTALITY OF ANASTATUS

During a severe winter in New England there is a large mortality of unprotected gipsy-moth eggs and of hibernating Anastatus. This fact was very evident in the large collections of gipsy-moth eggs which were made during the winter of 1917–18 to obtain Anastatus for colonization.

During this winter about 79 per cent of the Anastatus in the eggs under observation were killed. This figure was obtained from 52,800,000 gipsy-moth eggs, which contained 8,349,000 Anastatus larvae. Most of these eggs were collected at Peabody, Mass. The lowest temperatures for the winter recorded at the gipsy moth laboratory were observed on December 30, 1917, and February 2, 1918, when the readings were —14° and —15° F., respectively.

The collections of gipsy-moth eggs made south of Boston during the winter showed a much lower mortality of Anastatus, and in some areas there was practically no winter killing.

To determine the winter mortality of Anastatus, collections of gipsy-moth eggs were made during the spring of 1922 and 1923 from points scattered over the area where it was well established. The collections showed

for the entire area an issuance of 67.2 per cent of the Anastatus in 1922 and 79 per cent in 1923. The laboratory thermograph at Melrose Highlands, Mass., recorded —15° F. in February, 1922, and —13° F. in February, 1923. Egg clusters collected below and above the snow line were kept separate, and the issuance of Anastatus recorded. It was found that of the eggs collected in 1922 the issuance from those below the snow line was 90.6 per cent, from those above the snow line 49 per cent, and the average of all was 67.2 per cent. Of the eggs collected in 1923 the issuance from those below the snow line was 82.2 per cent, from those above the snow line 76.3 per cent, and the average was 79 per cent.

Among the gipsy-moth eggs from which these figures were obtained were many collected from the northern part of the area colonized with Anastatus. The average mortality of Anastatus in the area south of Melrose is not nearly so great as it is north and northwest of this town. There are many winters with practically no mortality of Anastatus over the eastern half of Massachusetts, southeastern New Hampshire, and southwestern Maine.

During the spring of 1923 collections of gipsy-moth eggs were made from high land, as from the tops or near the tops of hills, and also from low land, as along the banks of rivers or brooks or the edge of a swamp or meadow. The eggs taken below the snow line and those from above it in each set of collections were kept separate. The percentages of issuance of Anastatus were, for the eggs taken from the high land, 85 for those above and 90 for those below the snow line, and for the eggs from the low land, 67 for those above and 88 for those below the snow line.

The collections indicate that there is considerably more winter killing of Anastatus in the low areas than in the higher ones, and that the snow in either location offers very good protection to Anastatus.

FIELD WORK TO OBTAIN ANASTATUS FOR COLONIZATION

The first collections of gipsy-moth eggs from New England to secure Anastatus for further colonization work were made in the fall of 1909. Most of the eggs were collected in the vicinity of the centers of four of the five colonies which had been established in the summer. Each season thereafter for some years collections of large numbers of gipsy-moth eggs were made at different colonies suitably located (Fig. 7, *a*). Using the exact point of liberation of the colony as a center, eight lines were run, four to the cardinal points of the compass and the other four intermediate to them. The first year or so collections of gipsy-moth eggs were made at 25-foot, 50-foot, and 100-foot intervals along the lines as far out from the center as Anastatus was found. In this manner the area was determined where the parasitism by Anastatus was high enough to warrant the general collection of gipsy-moth eggs to obtain Anastatus for colonization.

As Anastatus spread and the lines became longer, the greater part of the work was done in a colony convenient to the laboratory at Peabody, Mass. A chart of the area covered is shown in Figure 8. At this colony the lines were run farther from the center each year in order to determine the area from which to collect as many egg clusters as possible. General collection of eggs was made in the area where the eggs showed as high as 10 per cent parasitism. This work was not confined to the Peabody area alone, but for a number of years most of the material collected came from that area.

Year after year these lines continued outward until several of them extended 3 miles from the center. Collections of 10 egg clusters each were made at the center and at intervals of 100 feet along the lines for 600 feet; then at intervals of 100 yards as far as the collections showed parasitism by Anastatus. These sample collections were examined at the laboratory and the percentage of parasitism in each case was ascertained. From the data thus acquired the area for general collection of eggs was determined. The collections made in 1915 and later do not show the spread from the original colony, for in the spring of 1915 the outside sections of Peabody and the surrounding towns were colonized with Anastatus and some of this newly colonized area was included within the 10 per cent limit.

The increase year by year in the area which included the points showing a parasitism of at least 10 per cent, and in which most of the egg masses were collected, is here presented:

Year	Acres occupied
1910–11	1. 7
1911–12	2
1912–13	12
1913–14	234
1914–15	242
1915–16	903
1916–17	2, 261
1917–18	3, 915
1918–19	8, 008
1919–20	8, 198

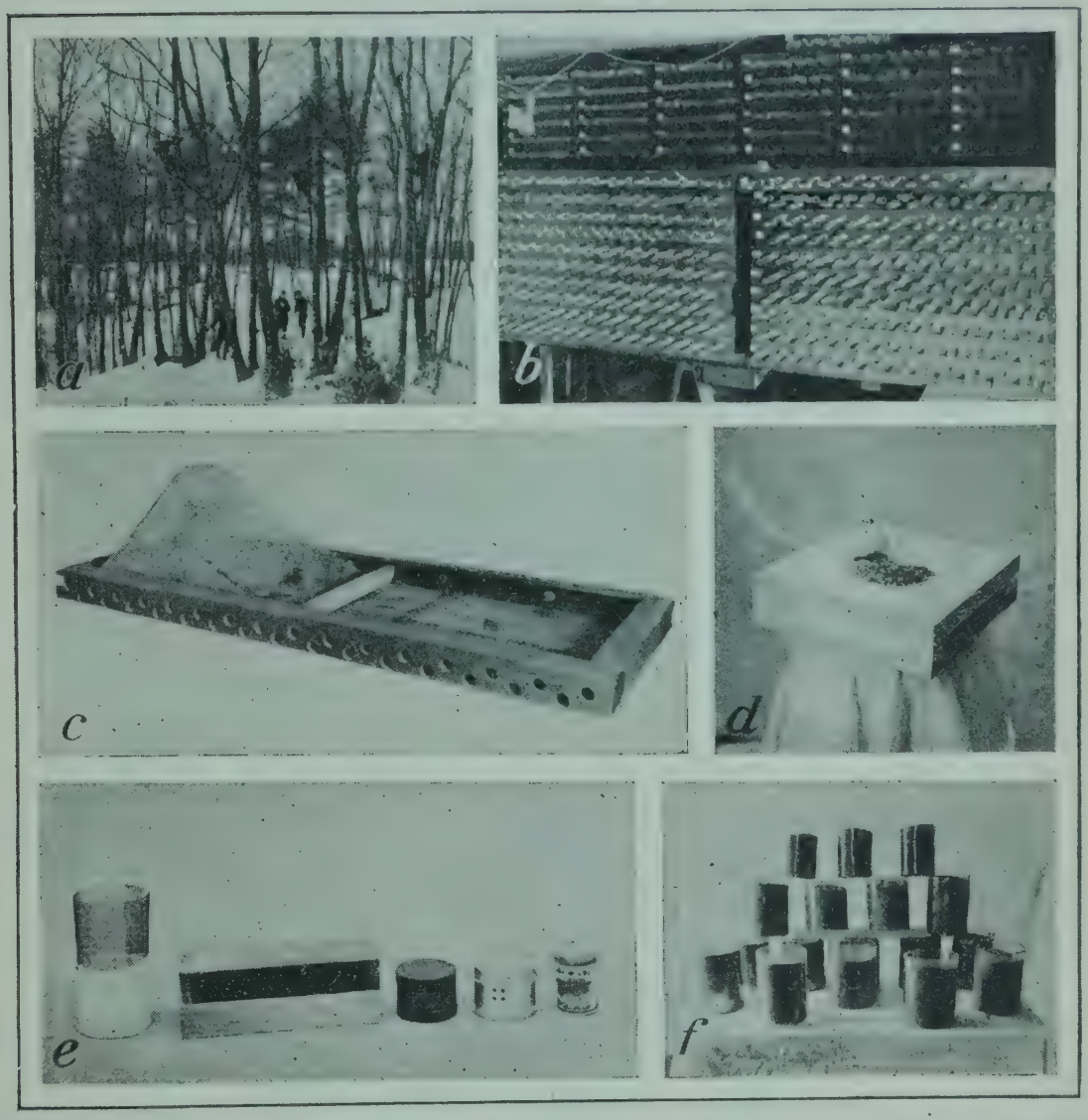

FIG. 7.—*Anastatus bifasciatus* and its colonization: *a*, Collecting gipsy-moth eggs to obtain Anastatus for colonization; *b*, trays used for hatching the nonparasitized gipsy-moth eggs; *c*, interior of tray after the hatching of the gipsy-moth eggs is complete (mosquito netting full of webbing left by first-stage gipsy-moth larvae before they entered the glass tubes and were removed); *d*, hand-sifting tray; *e*, types of colonization cages for Anastatus, the two on the right being the most satisfactory; *f*, six bushels of gipsy-moth eggs after having passed through the separating apparatus, the eggs being in the proper condition to be run through the bouncing apparatus. In *b* and *c* are shown methods used in preparing Anastatus for colonization before the apparatus shown in Figure 9 was developed

The extent of this work has varied in different years. In some seasons 5 to 6 bushels of gipsy-moth eggs have been collected, and one winter about 9 bushels were gathered. The greatest number of eggs handled at the laboratory in one season amounted to more than 98,000,000 in the winter of 1918–19. On an average, about 10 per cent of these were parasitized.

For the first few years after Anastatus became established, most of the gipsy-moth egg clusters were collected near the centers of the few colonies which had been liberated. The eggs were so kept as to allow the nonparasitized gipsy-moth eggs to hatch, and the remaining material was divided for colonization in the spring.

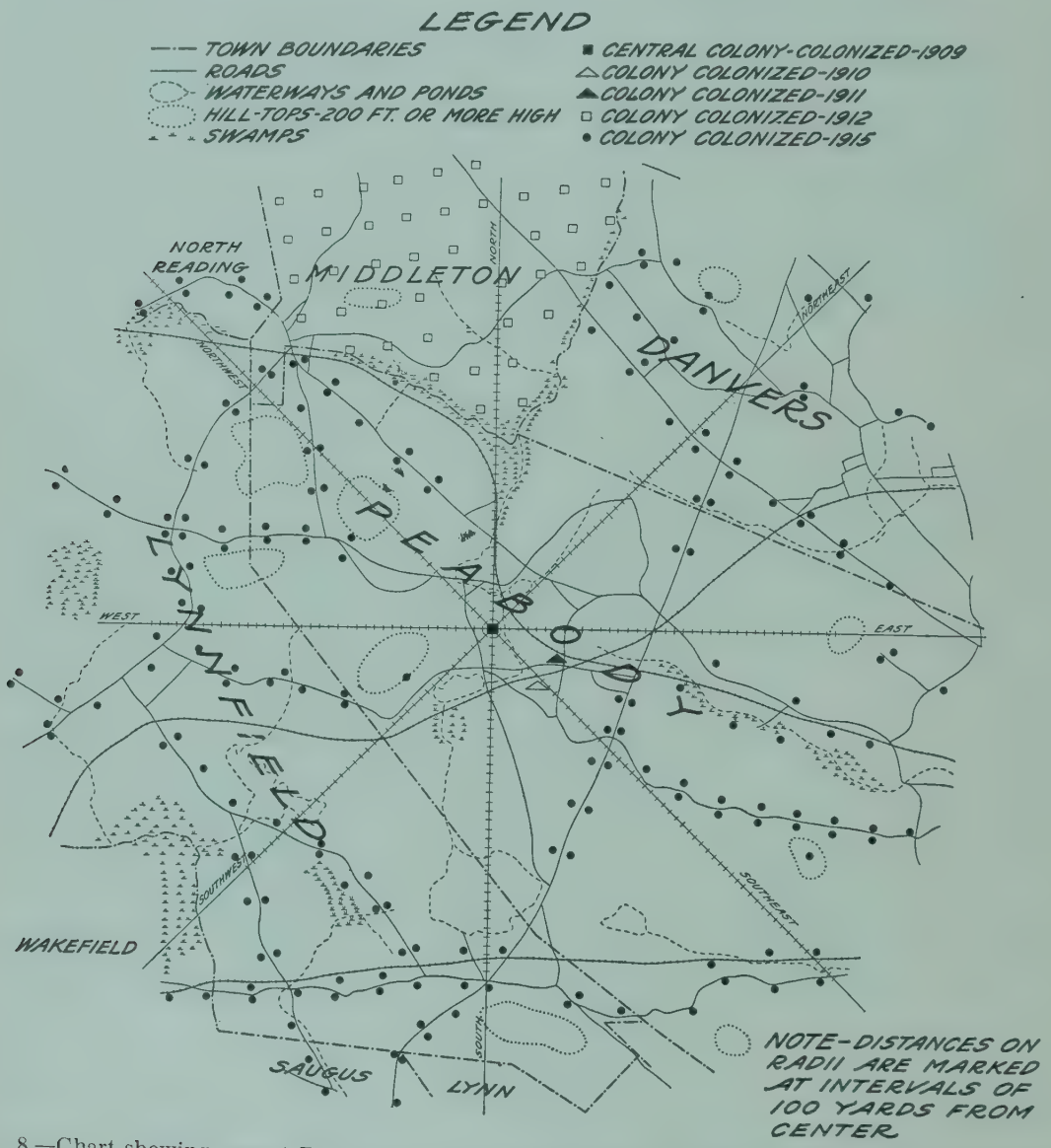

FIG. 8.—Chart showing area at Peabody, Mass., where some of the records of dispersion and percentage of parasitism by Anastatus were obtained. The checks on the eight lines are 100 yards apart and show the points where the sample collections of gipsy-moth eggs were made. The different marks on the chart show the location of Anastatus colonies liberated in this area during the different years. Scale, 1 inch= 1.7 miles

LATER LABORATORY METHODS USED IN HANDLING GIPSY-MOTH EGGS TO OBTAIN ANASTATUS BIFASCIATUS FOR COLONIZATION

Each fall small samples of gipsy-moth eggs from different locations are collected and examined to determine the percentage of parasitism by Anastatus. To obtain Anastatus for colonization, large collections of egg masses are then made at the points where the parasitism is 10 per cent or more.

The collections of egg masses increased from year to year until as many as 9 bushels of gipsy-moth egg clusters were collected and handled in one season at the laboratory. The methods used to separate the eggs containing parasites from the rest of the material have varied considerably and have been gradually improved until at present much of the work is practically mechanical. These improvements have resulted in a large saving of time and expense.

In order to handle the eggs in a satisfactory manner it was necessary first to separate them from the mass of hairs in which the female moth had packed them. This was originally done by rubbing the egg clusters with the bare hand or with a pad over a piece of cotton cloth, drawn tightly over a tray, as in Figure 7, *d*, until the hair had been rubbed through the cloth, leaving only the eggs on the cloth. A machine on the principle of the gristmill is now used to do this sifting. This machine, shown in Figure 9, *a* and *b*, has two disks about 3 feet in diameter, the lower one of which is stationary, while the upper revolves, the power being furnished by a small motor. The inner surface of each disk is padded with felt covered by canvas. The upper disk rests on a spindle, and can be adjusted so as not to injure the eggs. The broken egg clusters are fed into the machine through a boxlike opening in the upper disk; as this disk revolves the eggs are gently rubbed between the two inner surfaces. As the eggs are worked to the circumference of the disks they become separated, cleaned, and drop off, assembling in a jar below (fig. 9, *a*). The hair and dust from the egg clusters are removed by means of a blower attached to the center of the apparatus.

This apparatus is not only a great labor saver, but by cleaning the dust and hair from the eggs it eliminates to a large extent the irritation of the nose and throat, which has at times severely affected most of the men at the laboratory.

After the eggs have been cleaned (fig. 7, *f*) they are ready for the separation of the parasitized eggs. Formerly the eggs were placed in trays (fig. 7, *b*) until the hatching of the nonparasitized eggs was completed. The cleaned eggs were spread evenly over the bottoms of the trays. In the front of each tray are numerous 1-inch holes, the use of which will be explained later. In each tray, on top of the layer of eggs, was placed one or two thicknesses of cloth mosquito netting, through which the small caterpillars had to crawl on leaving the tray. A considerable part of the web spun by the crawling larvae was caught in this netting (fig. 7, *c*). When the eggs and netting were in place each tray was entirely covered with black paper and placed on racks in a warm room to allow the nonparasitized eggs to hatch. Before it was time for the eggs to hatch the paper was broken over the holes in the front of each tray and glass vials were inserted (fig. 7, *b*), into which the caterpillars crawled. When hatching began the entire attention of several men for nearly two weeks was required to remove the larvae from the glass tubes. This was best accomplished by cleaning out each tube with a camel's-hair brush and placing the caterpillars in kerosene. This method was later much improved upon by using a vacuum cleaner with a special attachment which fitted into the tubes.

After hatching was completed the black-paper tops were taken from the trays and the mosquito netting was removed, with much of the webbing left by the crawling caterpillars. The remaining material was separated as well as possible by allowing the air from an electric fan to pass over it, blowing out some of the eggshells and light, dead eggs. This separating process was greatly improved by the development of a machine (fig. 9, *d*) so constructed that as the parasitized eggs, dead eggs, and eggshells ran down a chute the dead eggs and eggshells were drawn off by suction, while the parasitized eggs, which are heavier than the other material, assembled in a glass tube at the bottom of the apparatus.

This method of allowing the nonparasitized gipsy-moth eggs to hatch so that the parasitized eggs could be separated from the rest of the material was a long and tedious process and has been discontinued except for small experiments. Now a simple apparatus called a bouncing machine (fig. 9, *c*) is used. The eggs are allowed to run down a wooden chute, at the bottom of which is a small piece of tin placed at right angles to the inclined surface. This piece of tin is termed a "take-off"; when the eggs hit it they bounce into the compartments of a box beneath. The eggs containing parasite maggots do not have the same capacity for bouncing as the healthy gipsy-moth eggs and therefore fall into the first and second compartments, while the other eggs bounce farther and drop into the compartments beyond. The nonparasitized eggs are then destroyed. The eggs containing parasites are put into small manila envelopes in lots of about 1,000 parasites each for colonization in the spring. These parasitized eggs are not actually counted, but are measured in a small glass tube so graduated that each colony will contain 1,000 eggs.

COLONIZATION OF ANASTATUS

The first colonization of Anastatus in America was made in 1908, when 513 adults were liberated. Most of these

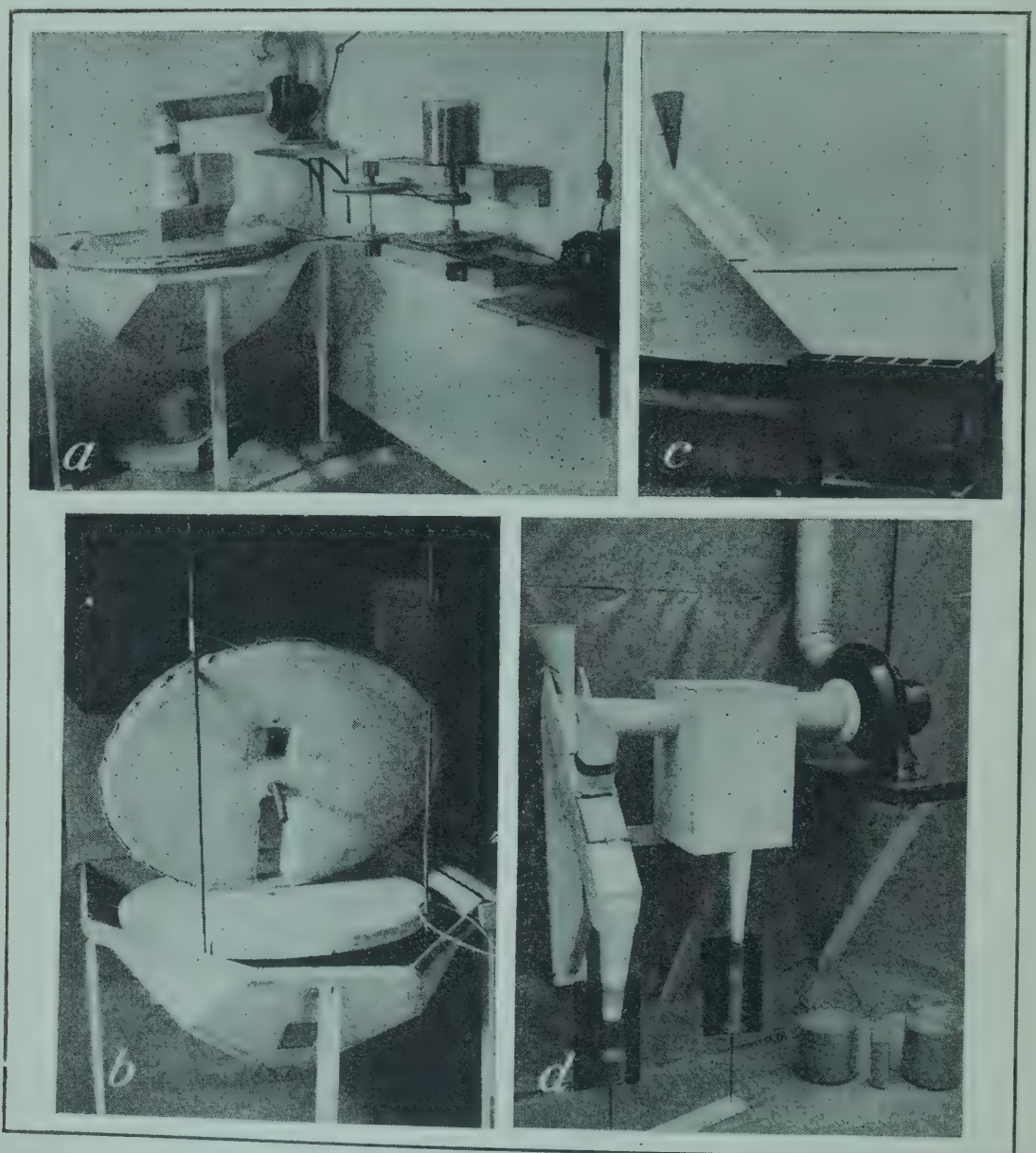

FIG. 9.—Apparatus used in colonization of egg parasites of the gipsy moth: *a*, Separating apparatus, used to separate gipsy-moth eggs from the hair; *b*, showing inner surfaces of disks of separating apparatus; *c*, bouncing apparatus, used to separate nonparasitized gipsy-moth eggs from eggs containing Anastatus larvae; *d*, winnowing apparatus, used for separating the eggs containing Anastatus larvae from the egg-shells, dead eggs, and other matter removed from the hatching trays

Anastatus were reared from gipsy-moth eggs collected in Russia and sent to the laboratory; a few came from Japanese eggs. In 1909 the Anastatus were colonized as mature larvae within the host eggs. Most of this material was received from Hungary, but it included about 5,000 Anastatus from Japan.

298 towns are in Massachusetts, 169 in New Hampshire, 97 in Maine, 35 in Vermont, 32 in Rhode Island, and 31 in Connecticut. The area which has been colonized by Anastatus is shown in Figure 10. No further colonization of Anastatus is necessary in 441 of these towns, so thoroughly has the work been done. In the other

TABLE II.—*Summary of the colonization of Anastatus bifasciatus*

	Massachusetts		New Hampshire		Maine		Rhode Island	
	Colonies	Individuals	Colonies	Individuals	Colonies	Individuals	Colonies	Individuals
1908	1	513						
1909	5	128, 180						
1910	97	105, 000						
1911	160	227, 500	26	26, 000				
1912	621	621, 000						
1913	851	851, 000	571	571, 000				
1914	1, 047	1, 047, 000	514	514, 000				
1915	6, 877	6, 877, 000	1, 501	1, 501, 000	813	813, 000		
1916	8, 218	8, 227, 000	3, 522	3, 522, 000	942	942, 000		
1917	1, 722	1, 722, 000	4, 376	4, 376, 000	1, 357	1, 357, 000	573	573, 000
1918 [a]	645	645, 000	575	575, 000	377	377, 000	93	93, 000
1919	1, 946,	2, 111, 000	6, 130	6, 273, 000	1, 479	1, 675, 000	69	135, 000
1920			1, 154	1, 214, 000				
1921	2	[b] 500, 000						
1922	1, 854	3, 708, 000	408	816, 000	121	242, 000	129	258, 000
1923	333	333, 000	225	225, 000	167	167, 000		
Total	24, 379	27, 103, 193	19, 002	19, 613, 000	5, 256	5, 573, 000	864	1, 059, 000

	Connecticut		Vermont		Total	
	Colonies	Individuals	Colonies	Individuals	Colonies	Individuals
1908					1	513
1909					5	128, 180
1910					97	105, 000
1911					186	253, 500
1912					621	621, 000
1913					1, 422	1, 422, 000
1914					1, 561	1, 561, 000
1915					9, 191	9, 191, 000
1916					12, 682	12, 691, 000
1917	51	51, 000			8, 079	8, 079, 000
1918 [a]	55	55, 000			1, 745	1, 745, 000
1919	144	144, 000			9, 768	10, 338, 000
1920					1, 154	1, 214, 000
1921					2	500, 000
1922	215	430, 000	56	112, 000	2, 783	5, 566, 000
1923	200	200, 000	5	5, 000	930	930, 000
Total	665	880, 000	61	117, 000	50, 227	54, 345, 193

[a] Enough eggs of the gipsy moth were collected this year to obtain from them 8,000,000 Anastatus. The winter of 1917–18 was very severe, with heavy mortality of both host and parasite.
[b] Estimated. The greater part of the material was used for a large reproduction experiment.

Table II presents statistics of the yearly colonization. Since 1909 all Anastatus which have been liberated have been obtained by collecting gipsy-moth eggs in New England. As a rule, each colony has contained 1,000 Anastatus. They have been put out as full-grown larvae within the gipsy-moth eggs.

Over 54,000,000 Anastatus have been liberated in New England, distributed over 662 towns. Of these, 221 towns the colonies were liberated rather sparsely, as the gipsy-moth infestations at the time of colonization were light and scattering. It will be necessary to place more colonies of Anastatus in many of the partially colonized towns.

During the winter months the Anastatus which are to be colonized in the spring are gathered from the field, in the gipsy-moth eggs. After separating the parasitized from the healthy

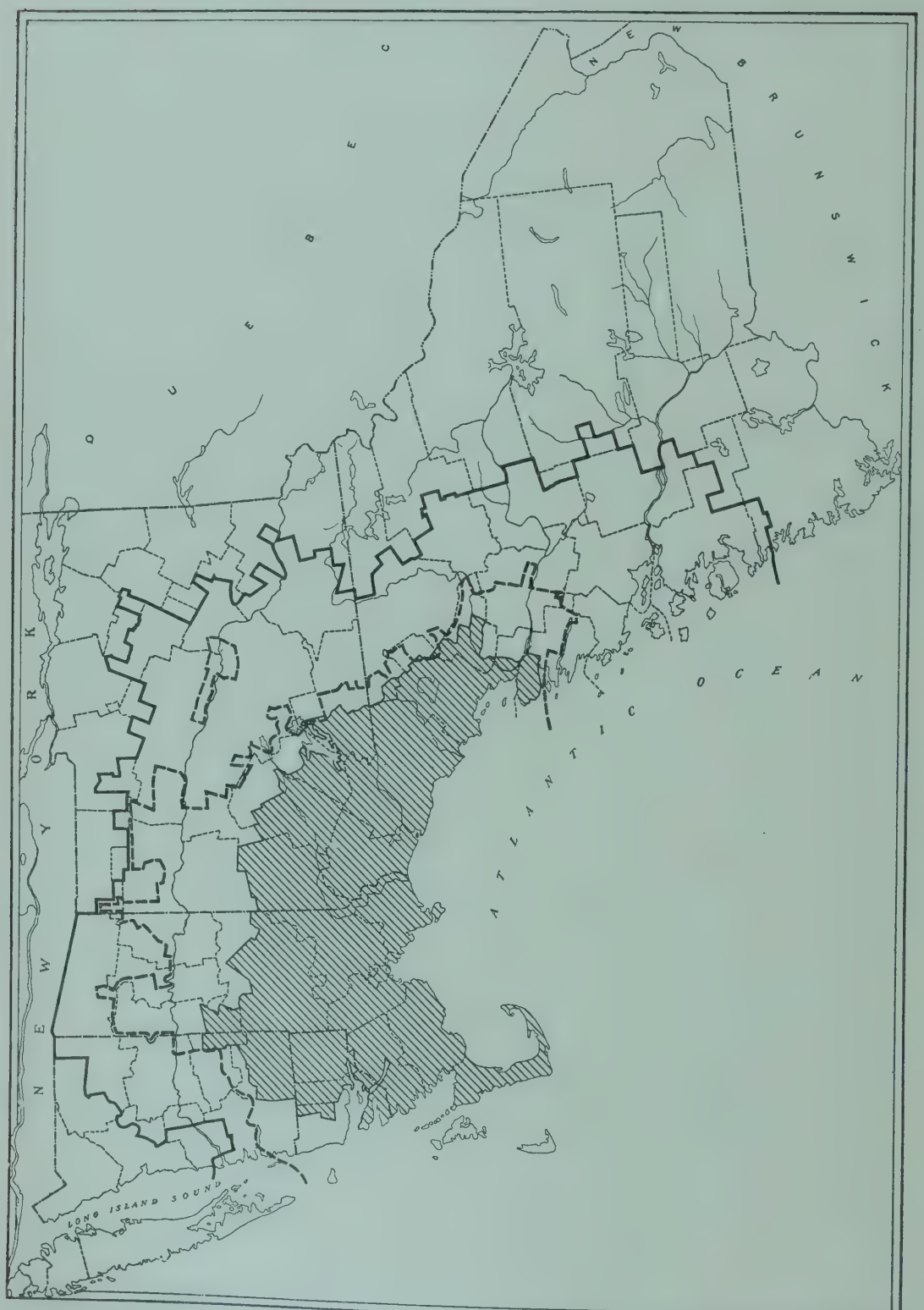

FIG. 10.—Map showing colonization of *Anastatus bifasciatus* in New England. The heavy black line is the quarantine line for the gipsy moth, the area to the right of this line being infested by that insect. The shaded area has been solidly colonized with Anastatus. The area between the broken line and the shaded area has been partially colonized with Anastatus

gipsy-moth eggs 1,000 parasitized eggs are put into small manila envelopes and stored in a cool place until the roads can be traveled in the spring. In some seasons the work of colonization has required a considerable force of men and auto vehicles in order to liberate the colonies in the limited time available. In several cases the various States have assisted greatly in the work.

The men are supplied with large blue-print maps (fig. 11) showing all roads in the towns to be colonized. Each man is given a supply of colonies and small tin cans (fig. 7, *e*, at right). Each can has three ⅛-inch holes in its side near the top. All of the roads in each town are traversed and a colony of 1,000 Anastatus is liberated on each side of the road at intervals of a quarter of a mile, if there is woodland area with sufficient gipsy-moth infestation to warrant such frequency. The colonies are placed 100 to 200 feet from the roadside. The can is nailed to a tree trunk in an inconspicuous place (fig. 2, *f*) and the parasitized eggs are poured into the can. A tight-fitting cover is placed on each can to prevent the rain and birds from destroying the colony. The location of each colony is marked on the blueprint map of the town (fig. 11). In each town two colonies in easily accessible locations are marked in the field by painting with white paint on a roadside tree a letter "A," with arrows pointing to the center of the colony (fig. 2, *c*). A note for filing at the laboratory is written, giving directions so that the marked colonies can be easily located later.

SUCCESS OF COLONIES AND DISTRIBUTION OF ANASTATUS

Collections of gipsy-moth eggs for data in regard to the establishment of Anastatus after colonization have shown that this parasite is easily established and recovered. Eighty to 90 per cent of the colonies liberated in Massachusetts, where collections of host eggs have been made later, have given proof of its presence.

About 50 per cent of the collections of gipsy-moth eggs which have been made in the other New England States showed the presence of the parasite. Anastatus has been recovered from 161 towns in Massachusetts, 75 in New Hampshire, 20 in Maine, 8 in Rhode Island, and 2 in Connecticut, or 266 in all.

Anastatus are now being found in gipsy-moth eggs sent to the laboratory from practically every town where the species has been thoroughly colonized. Figure 5 shows the area from which Anastatus has been recovered. It should be noted that the species is much more hardy than Schedius and is being recovered from the towns in the northern part of the colonized area.

PERCENTAGE OF ISSUANCE OF ADULT ANASTATUS FROM THE COLONIZATION CANS

The contents of many colonization cans have been examined at the laboratory. Cans showing a low percentage of issuance of Anastatus adults have occasionally been found. More than half of the cans which have been brought to the laboratory have shown that over 90 per cent of the parasitized eggs had yielded Anastatus adults. The average percentage of issuance is 68 for all the cans which have been collected.

DISPERSION

Very careful records were kept of the spread of Anastatus during the first years after its establishment in Massachusetts. Gipsy-moth egg clusters were collected along lines run in eight directions from the centers of several colonies. At first the egg collections were made at 50-foot intervals. Later, as the spread became greater, the collections were made along the lines at intervals of 100 feet for the first 600 feet, and then of 100 yards to points well beyond the recovery of the parasite. The examination of the eggs collected showed for the first year a spread of about 200 feet, and for the second, 500 feet.

Similar collections were made in the following years until later liberations of parasite colonies interfered with the data desired. These collections showed that after a colony became well established the annual spread measured from its center was very much greater than it was in the first year or two. The data were obtained from five widely separated towns, and the spread was similar at each of the five colonies under observation. An amazing increase in the rate of dispersion was noted as year succeeded year and the colonies became stronger. For the first year after the five colonies were liberated the spread from each center was 200 feet, and for the second year 500 feet. For the third year the average spread was 890 feet, and the extreme spread

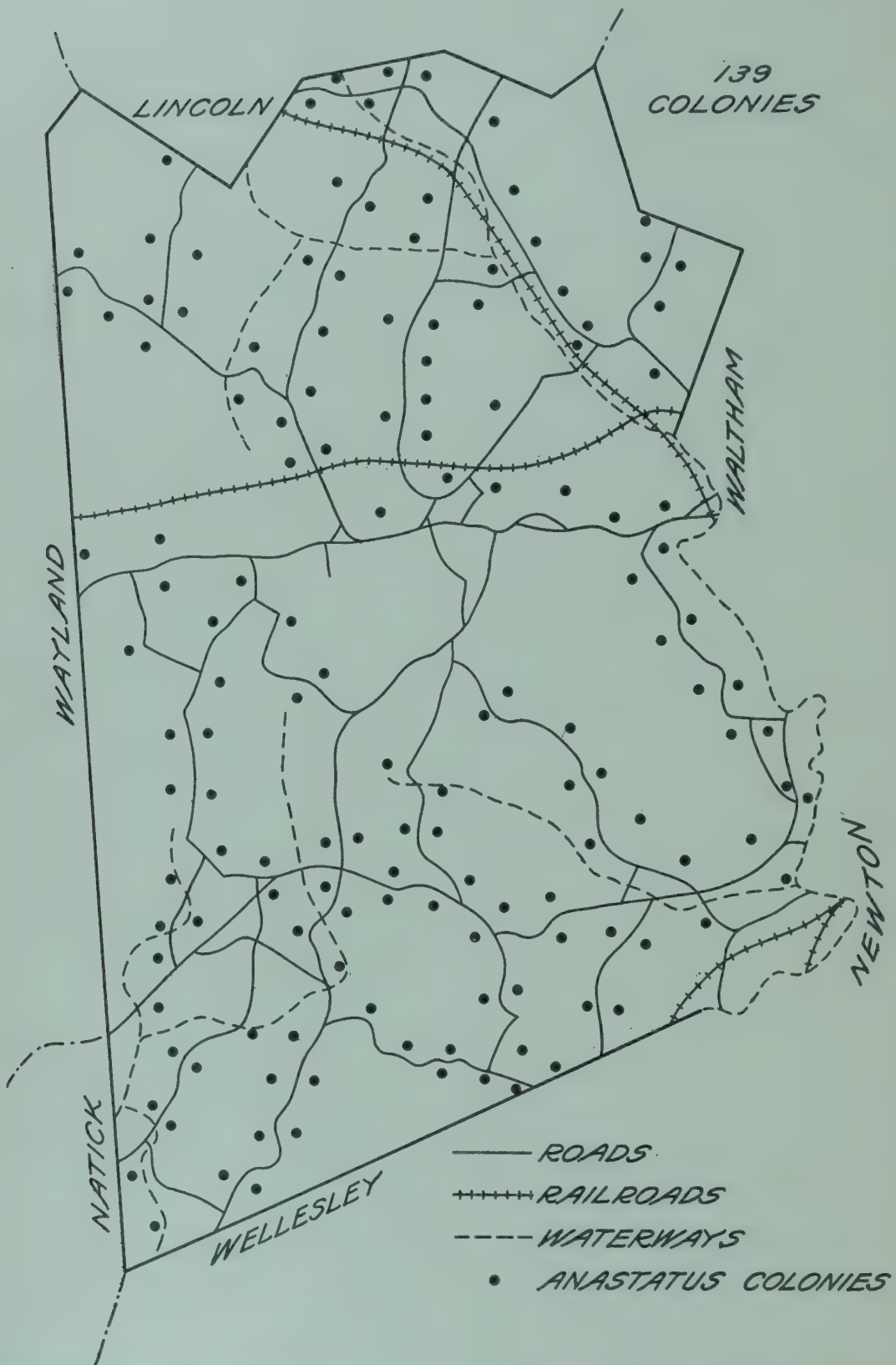

FIG. 11.—Copy of blue-print map of town showing location of Anastatus colonies. Each dot represents a colony of 1,000 Anastatus. Scale, 1 inch=4.35 miles

1,100 feet. For the fourth and fifth years the averages were 2,520 and 4,650 feet, and the extreme spreads 3,600 and 4,800 feet, respectively.

There seemed to be very little evidence in these collections to indicate that Anastatus spread more rapidly in one direction than in another. The spread to the north and northwest was greatest in some colonies, but other colonies showed the greatest dispersion in the opposite direction. The wind probably is an important factor in the dispersion of this parasite, for the female is not known to fly, although it has great jumping ability, and if a gentle wind stirs it into activity, causing it to jump often, it would without doubt be carried considerable distances. The nature of some of the recoveries indicates strongly that the females are blown distances of 300 and 400 yards, for several collections of gipsy moth eggs made along the same line a hundred yards apart often show no presence of Anastatus, although the species will be found present in the eggs collected farther from the center.

PERCENTAGE OF GIPSY-MOTH EGGS PARASITIZED BY ANASTATUS

In order to ascertain the amount of parasitism of the gipsy-moth eggs by the introduced parasites, collections of the host eggs have been made each year from many points over the infested area. The percentage of parasitism by Anastatus which has been found in a majority of the collections is very encouraging.

The gipsy-moth eggs collected from the colony sites usually show the presence of Anastatus the year after colonization. In such cases the amount of parasitism is small, but it increases year after year until the maximum is reached. Because Anastatus spreads only short distances some gipsy-moth eggs which are collected in the newly colonized territory, but not from colony sites, often show no parasitism.

Very many collections of host eggs have been made to determine the percentage of parasitism. This work has been carried on most intensively in the areas which have been colonized longest, and for the most part at Peabody, Mass., and neighboring towns.

Each sample collection of eggs contained, when possible, 10 egg clusters. A collection was made at the center and along eight lines, from the center to the four cardinal and the four intermediate points. The collections within 600 feet of the center were made at intervals of 100 feet, and at greater distances at intervals of 100 yards.

The collection of gipsy-moth eggs was carried on intensively at Peabody from 1910 until the spring of 1921. The parasitized eggs obtained were used for new Anastatus colonies. The information obtained by the examination of these sample collections was found useful in determining the area over which it would be practical to do general collecting to obtain Anastatus for colonization.

A summary of the percentage of parasitism obtained from the Peabody collections is presented in much abbreviated form in Table III. Figure 8 shows the points where the egg collections were made.

At Peabody the first colony of Anastatus was liberated in 1909. In 1910 another colony was liberated, and a third in 1911. The 1910 colony was liberated about 1 mile southeast, and the 1911 colony about one-fourth mile nearly east, of the 1909 colony.

TABLE III.—*Parasitism of eggs of the gipsy moth collected at and near Peabody, Mass., for the specified years and distances from center of colony*

Year eggs collected	At center	From center to 600 feet	From 200 yards to 1,400 yards	From 1,400 yards to 2,300 yards	From 2,300 yards to 3,600 yards	From 3,600 yards to 4,500 yards	From 4,500 yards to 5,000 yards
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1910–11		18					
1911–12	33	19	1				
1912–13	17	17					
1913–14	31	28	8				
1914–15	20	21	9				
1915–16	29	27	17	6	3	1	1
1916–17	32	26	19	12	7	1	3
1917–18	26	22	24	18	16	17	18
1918–19	4	19	20	18	21	15	17
1919–20	19	20	17	16	14	13	16
1920–21	29	27	18	22	17	17	18

No more Anastatus were liberated in this area until the spring of 1915, when the outside area of Peabody and the surrounding towns were solidly colonized with Anastatus. The effect of this later colonization is shown in the evenness of parasitism manifested in the collections made 2 and 3 miles from the center of the original Peabody colony.

After 1921 the gipsy-moth infestation in this area was too light for general collection of eggs to obtain Anastatus for colonization. In each of the following years sample collections of gipsy-moth eggs when possible of 10 egg clusters each, have been made to determine the percentage of parasitism in this area. About 40 collections were made at points scattered over the area where general collecting was previously done. The average percentage of parasitism of these eggs by Anastatus has been, for 1921–22, 33; for 1922–23, 23; and for 1923–24, 18.

In order to determine the average percentage of parasitism by Anastatus of the gipsy-moth eggs in a town which should be representative several years after colonization, 10 egg masses were collected from about 50 points in the town of Weston, Mass. The egg collections were made in the woodland near the roadside or from roadside trees. Each road in the town was included in the survey. The average percentage of parasitism of the gipsy-moth eggs for each year during the time covered by the survey was, for 1917–18, 21; for 1918–19, 19; for 1919–20, 13; for 1920–21, 33; for 1921–22, 34, and for 1922–23, 21.

During the last five years representative collections of gipsy-moth eggs have been made from the following five towns in Massachusetts: Burlington, Dover, Lynnfield, North Reading, and Wilmington. About 17 points were chosen in each town and collections of 10 egg clusters when possible were made at each location. The average percentages of parasitism of the gipsy-moth eggs by Anastatus for the five years in each town are shown in Table IV.

The figures for the year 1921–22 show the maximum percentage of parasitism of gipsy-moth eggs by Anastatus which has been obtained over a large area in Massachusetts since the establishment of this parasite in New England.

TABLE IV.—*Average percentage of parasitism of gipsy-moth eggs by Anastatus bifaciatus as indicated by representative collections made in five towns in Massachusetts*

Year	Burlington	Dover	Lynnfield	North Reading	Wilmington
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1919–20	14	14	11	16	16
1920–21	24	34	19	23	26
1921–22	35	37	31	35	34
1922–23	32	31	28	25	30
1923–24 [a]	28	24	14	11	18

[a] The collections of 1923–24 were made somewhat earlier than usual, possibly before some of the Anastatus were through ovipositing; the percentage of parasitism is therefore thought to be rather low.

PERCENTAGE OF PARASITISM BY ANASTATUS AND SCHEDIUS OF GIPSY-MOTH EGG CLUSTERS AT VARIOUS HEIGHTS IN THE TREES

Collections of gipsy-moth eggs to determine the percentage of parasitism by Anastatus and Schedius of the eggs at different heights in the trees have shown that the parasitism is approximately the same over the entire tree. The collections of eggs for data on Anastatus were made in four separate locations. The egg clusters collected from the trees at distances of from 1 foot to 6 feet from the ground were termed "low-point" collections; those collected at heights of from 20 to 55 feet were termed "high-point" collections. Five egg clusters were collected at the low and five at the high points from each of the trees selected in the four locations. Table V contains a summary of the examinations of the eggs.

The average percentage of parasitism of the eggs collected from the low points was 37.0, and for those from the high points 35.8. The difference between the average size of the lower egg clusters and that of the higher could account for this difference, the higher being slightly the larger. Studies of the percentage of parasitism of egg clusters of different sizes have shown that the percentage of parasitism increases as the size of the cluster decreases.

TABLE V.—*Percentage of parasitism by Anastatus bifaciatus of gipsy-moth egg clusters collected at different heights in the trees*

Distance from ground eggs were collected	Number of egg clusters	Percentage parasitized by Anastatus	Average number of eggs in each cluster
1 foot to 6 feet	40	40.2	202
1 foot to 6 feet	40	30.7	273
1 foot to 6 feet	40	39.7	165
1 foot to 5 feet	40	37.5	205
Average		37.0	211
35 feet to 40 feet	40	35.5	280
20 feet to 35 feet	40	28.2	326
30 feet to 55 feet	40	42.3	127
35 feet to 40 feet	40	37.1	237
Average		35.8	242

Similar collections of gipsy-moth eggs have been made in areas where Schedius was abundant, to determine its parasitism of the eggs on various parts of the trees. Only a slight difference was found in the activity of Schedius at different heights, the average percentage of parasitism at less than 6 feet from the ground being 26, and that for heights of from 30 to 55 feet 29. This difference could be explained by the fact that the higher egg clusters averaged only 154 eggs each, while those collected at heights below 6 feet averaged 171 eggs.

PERCENTAGE OF PARASITISM BY ANASTATUS AND SCHEDIUS OF GIPSY-MOTH EGGS COLLECTED AROUND OBSERVATION POINTS

During 1911 and 1912 about 250 points, scattered over a considerable part of the area at that time infested by the gipsy-moth, were chosen and called "observation points." A tree at the center of each point was marked and a circle 100 feet in diameter was described around it. Careful records of the condition of the growth and intensity of the gipsy-moth infestation at each point have been kept for each year.

From 1912 through 1923 collections of gipsy-moth eggs have been made around many of these points. In each case an attempt was made to have a typical sample of eggs from which to determine the amount of egg parasitism. The eggs were collected just outside the 100-foot circle in eight different directions from the center. Two large, two medium, and two small egg clusters were collected at each of the eight places, a total of 48 egg clusters for each observation point. Each cluster was separately examined at the laboratory. The results are presented in Table VI.

The area covered by the observation point collections is shown in Figure 4. It will be seen that the eastern part of Massachusetts and the southeastern part of New Hampshire are well represented. The egg collections were not made with reference to parasite colonizations and the amount of parasitism shown is therefore representative for the entire area covered by those points.

A study of Table VI shows that the smaller the egg cluster the more heavily parasitized it is. The female Anastatus is unable to oviposit in all the eggs of a cluster, and the percentage of eggs parasitized decreases as the size of the cluster increases. When Anastatus is very abundant and several females are present on a gipsy-moth egg cluster as it is being laid, the parasitism increases, this being especially true in the case of small clusters. It will be noted that during the last two years this difference has increased materially, the small egg clusters being parasitized by over 10 per cent more than were the large ones.

The table shows a steady increase in parasitism by Anastatus until the fall of 1922, when a slight drop occurred. Schedius increased gradually through the fall of 1917, but with few exceptions has not been able to increase in this area since the severe winter of 1917–18.

PERCENTAGE OF GIPSY-MOTH EGGS PARASITIZED BY SCHEDIUS

It appears from the results shown in the foregoing table that Schedius is an unimportant factor as an egg parasite of the gipsy moth in the area represented. The climate of this area has been too severe during the period covered by the collections for this parasite to be of general abundance. During this period, however, Schedius has for several different years been an important egg parasite of the gipsy moth in numerous locations. The fact that it produces several generations each year allows it to increase rapidly under favorable conditions, and within the area under discussion collections of gipsy-moth eggs are often made which show from 10 to 20 per cent of the eggs killed by this parasite.

Schedius seems better suited to the milder climate of southern Massachusetts. For the last five or six years it has been increasing in abundance in

TABLE VI.—*Summary of examinations of gipsy-moth eggs collected around the "observation points"*

Year	Number of points of collection	Number of clusters collected				Eggs parasitized						Total parasitized—		Total parasitism
						By Anastatus—			By Schedius—					
		Large	Medium	Small	Total	Large clusters	Medium clusters	Small clusters	Large clusters	Medium clusters	Small clusters	By Anastatus	By Schedius	
						P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1912–13	77	1,131	1,127	1,102	3,360	0.207	0.19	0.28	0.33	0.50	0.64	0.21	0.43	0.64
1913–14	73	1,068	1,090	1,053	3,211	.718	.87	.23	1.09	1.19	1.54	.85	1.20	2.05
1914–15	69	1,069	1,067	1,048	3,184	.93	1.13	.93	.83	.97	1.16	.99	.93	1.92
1915–16	66	1,037	1,050	1,046	3,133	2.33	2.86	2.91	1.36	1.21	1.57	2.62	1.35	3.97
1916–17	64	921	1,015	1,001	2,937	4.36	5.27	6.46	2.36	2.77	3.31	5.01	2.66	7.67
1917–18	68	792	1,034	981	2,807	6.19	7.69	9.54	2.30	2.83	4.18	7.26	2.78	10.04
1918–19	64	890	913	852	2,655	9.25	10.22	10.75	.11	.09	.21	9.83	.12	9.95
1919–20	49	439	730	731	1,900	9.75	11.16	12.53	.50	.58	.92	10.84	.61	11.45
1920–21	49	667	742	694	2,103	15.57	17.85	19.56	.03	.07	.13	16.98	.06	17.04
1921–22	51	357	708	695	1,760	25.03	28.60	31.20	.09	.12	.23	27.86	.13	27.99
1922–23	49	351	758	333	1,442	17.22	24.25	30.10	.98	.85	1.12	21.94	.93	22.87
1923–24 [a]	17	119	359	104	582	18.51	23.02	30.35	.71	.52	.17	22.18	.55	22.73
1923–24 [a]	60	560	1,403	389	2,352	16.32	20.00	26.78	.33	.32	.21	20.96	.29	21.25

[a] A uniform method of collecting and examining the eggs was followed during the years from 1912 through the spring of 1923. The infestation was so light over much of the area during the fall of 1923 and spring of 1924 that the collections had to be made over a larger area around some of the points. The examination of the eggs from 17 of the points was made in the usual manner. A slightly different method was used for the examination of the eggs from 60 points for 1923–24; the results for the two methods are separately presented.

most of the towns on Cape Cod and along the northwest coast of Buzzard's Bay. Good records of parasitism by Schedius are often obtained from gipsy-moth eggs collected at Martha's Vineyard and from the northeastern part of Rhode Island.

Each fall a survey is made of the towns in the southern part of Massachusetts and collections of masses of gipsy-moth eggs are made from roadside and woodland trees. The percentage of parasitism by Schedius of these collections has increased each year to and through the fall of 1922. The examination of the eggs collected in the fall of 1922 gave the following percentages of parasitism for the towns named: Sutton, 35; Dartmouth, 36; Marion, 37; Mashpee, 18; Harwich, 26; North Harwich, 46; and Woods Hole, 48. Collections of eggs made during the fall of 1923 showed the parasitism to have fallen a few points, but it is still considered good.

SUMMARY

Two egg parasites of the gipsy moth have been established in America. *Schedius kuvanae* Howard, a species which had never been described until the project of gipsy-moth and brown-tail moth investigations was started, was introduced from Japan, its only known habitat. The other species, *Anastatus bifasciatus* Fonsc., came from Japan and from several parts of Europe.

The biology of the two species varies greatly, Schedius having several generations and hibernating as an adult, while Anastatus has but one generation and hibernates as a full-fed larva within the egg of the gipsy moth. Both species are slow-spreading parasites and considerable colonization work remains to be done before they will have the same distribution as the gipsy moth in the Northeastern States.

Anastatus was established by liberating the imported parasites, and its colonization has been continued each year by collecting its host's eggs in New England and separating the parasitized eggs from those containing gipsy-moth larvae. A total of 54,345,193 Anastatus have been colonized.

Schedius was established by breeding a relatively few adults received from Japan through several generations until enough adults of the parasite were obtained to warrant making liberations. It has been colonized in each succeeding year by breeding it through several generations at the laboratory, after having obtained the breeding stock from gipsy-moth eggs collected in New England. A total of 20,799,537 adult Schedius have been colonized.

The data which have been obtained and presented in this bulletin show that Anastatus is now an important egg parasite of the gipsy moth in the area in which it has been generally colonized in New England.

During the most favorable seasons Anastatus should be able to parasitize at least 35 per cent of the gipsy-moth eggs over a considerable portion of the infested area.

It is an important fact that the parasitism by Anastatus of the gipsy-moth eggs increases as the egg clusters decrease in size. There is a tendency for the egg clusters of the gipsy moth to decrease in size after years of great abundance. During a year of great abundance of this parasite, when several Anastatus females are ovipositing in the eggs, even while the egg cluster is being deposited, the average percentage of parasitism may run considerably higher than has yet been obtained.

Schedius is also proving to be an important parasite of the gipsy-moth eggs in the southern part of New England, but apparently it is not able to work to its best advantage in the more severe climates of the northern part of the territory infested by the gipsy moth.

In the southern area during favorable seasons Schedius should parasitize from 30 to 40 per cent of the eggs of the gypsy moth. The fact shown in laboratory experiments that Schedius can reproduce readily in the eggs of several species of insects in addition to those of the gipsy moth may at any time add to the value of this introduction.

NEW NEMATODES FROM NORTH AMERICAN MAMMALS[1]

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INTRODUCTION

The nematode worms described in this paper were collected from two species of mammals, the North American buffalo (*Bison bison* L.) and the North American beaver (*Castor Canadensis*), both of which are of considerable economic importance. The bison, once on the verge of extinction, is recovering itself to such an extent that it has been necessary to thin out the herd at Wainright, Alberta, Canada. Thanks are extended to S. Hadwen, of the University of Saskatchewan, for his kindness in submitting the collection of worms for study. The beaver, an important fur-bearing animal of this continent, has received little attention from parasitologists. Morgan[2] noted the presence of strongyles in the colon and cecum but did not give a description.

DESCRIPTION

NEMATODES FROM BISON

Dictyocaulus hadweni n. sp.

MALE: 52 mm. greatest diameter, 518 μ at about the middle, cuticle thin and smooth, without transverse striations. Head rounded, 89 μ in diameter, amphids conspicuous, other papillae not evident, tube leading from amphid reaching the nerve ring. Nerve ring about 311 μ, excretory pore about 620 μ, behind anterior extremity. Esophagus 1.48 mm. long; its width at the anterior extremity, excretory pore, and just before the intestinal valve, 89 μ, 104 μ, and 178 μ, respectively. Caudal bursa moderate in size, about 400 μ in diameter, nearly circular. Dorsal ray divided completely to base, abruptly trilobed at apex, the inner lobe slightly longer and thinner than the other two. Externo-dorsal ray simple, slender, not quite as long as either branch of dorsal ray. Postero-lateral and medio-lateral rays completely fused, forming a single slender ray slightly longer than the externo-lateral which is of the same thickness. Ventral rays separate to base or nearly so, ventro-ventral but about half as long as latero-ventral. Spicules stout, subacute at apices, each with a semicircular transparent wing in apical third, from 260 to 300 μ in length. Gubernaculum oval, feebly chitinized, about 85 μ long (fig. 1).

FEMALE: Length about 60 mm., greatest diameter about 640 μ, at the middle of the length. Pharynx about 100 μ deep, very narrow; esophagus 1.3 mm. long, its greatest diameter 192 μ, just before the intestinal valve. Nerve ring 340 μ. Excretory pore 475 μ behind the anterior extremity. Vulva transverse, lips prominent, situated at about the posterior third of the body length. Uteri divergent, the anterior branch reaching nearly to the esophagus. Anus 163 μ in front of the posterior extremity. Tail acute, conical. Eggs 80 by 35 μ, embryonated in uteri.

HABITAT: In lungs of *Bison bison*.

LOCALITY: Canada, Wainright, Alberta, February, 1923; S. Hadwen, collector.

TYPE: United States National Museum, Helminthological Collections, No. 26100; paratypes Nos. 25954 and 25956.

Compared with *D. filaria* (Rud.), the above species differs in the more abrupt termination of the branches of the dorsal ray, in the complete fusion of the postero-lateral and medio-lateral rays and in the longer spicules.

Besides the type material, there are two lots of worms in the helminthological collections of the United States National Museum from *Alce americanus* (No. 18832) and *Cervus canadensis* (No. 19456), respectively. While there are some slight differences between these and the specimens from the type host, they can hardly be considered as specific and they appear to be of the same species.

Ostertagia bisonis, n. sp

MALE: Length 6.2 mm., greatest width 80 μ, just in front of the bursa.

[1] Received for publication June 30, 1924; issued June, 1925.

[2] MORGAN, L. H. THE AMERICAN BEAVER AND HIS WORKS. 330 p., illus. Philadelphia. 1868.

Diameter of head 19 μ; diameter of body at nerve ring 45 μ, at base of esophagus 60 μ. Esophagus 620 μ in length; nerve ring 207 μ behind the anterior extremity. Excretory pore and cervical papillae at same level, 265 μ behind anterior end. Bursa similar in shape to that of *O. trifurcata* Rans., 178 μ long in lateral view, prebursal papillae short and inconspicuous, 16 μ in front of the origin of the ventral rays. Dorsal ray slender, 32 μ long from origin to bifurcation, each arm 36 μ long with a very short external branch near the tip. Externo-dorsal ray ends near the margin of the bursal membrane close to the limit of the thick dorsal level, each spicule gives forth two thin, nearly transparent, acute processes, directed posteriorly, each process about 29 μ long. Gubernaculum absent.

FEMALE: Length 6.75 mm., maximum thickness 89 μ, in vicinity of the vulva. Head 22 μ in diameter, esophagus 670 μ long; nerve ring 215 μ behind anterior end. Excretory pore and cervical papillae at same level, 280 μ behind anterior end. Vulva a transverse crescentic slit, nude, 1.17 mm. in front of posterior extremity. Anus 144 μ in front of tip of tail. Tail gradually attenuated, rounded at tip. Combined length of oviejectors about 450 μ; sphincters 45 μ long by 64 μ in diameter.

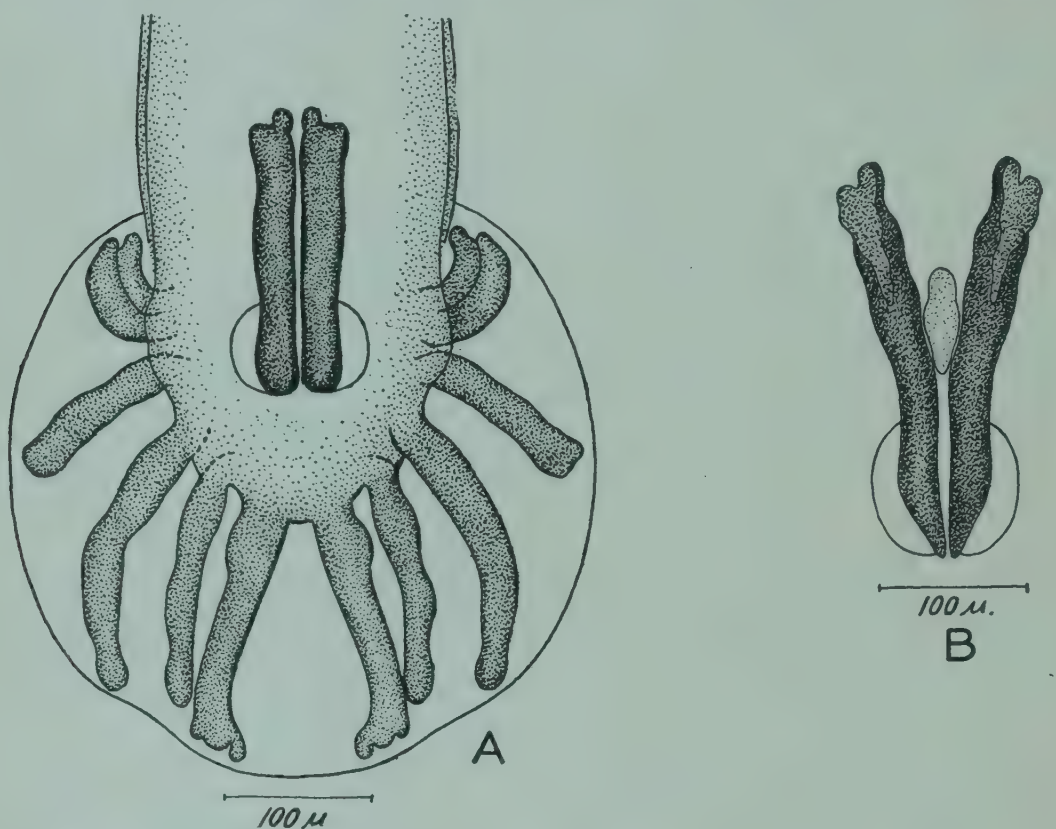

FIG. 1.—*Dictyocaulus hadweni*, n. sp. *A*, bursa of male; *B*, spicules and gubernaculum

lobe. Dorsal lobe 80 μ long, 48 μ wide and 45 μ thick, granular, not transparent as the bursal membrane. Tip of medio-lateral ray only half as far from that of postero-lateral as from that of externo-lateral. Latero-ventral ray bent so as to bring its tip in close proximity with that of the ventro-ventral. Spicules from 130 to 140 μ long, similar in length but differing in conformation. The right spicule ends in a short corkscrewlike hook, while the tip of the left is acute and slightly curved. On the ventral aspect of each, at about 42 μ before the posterior extremity there is a small boss, directed toward the median line of the worm. At the same

Eggs ovate, 64 to 77 μ long by 45 to 48 μ wide.

HABITAT: In fourth stomach and duodenum of *Bison bison*.

LOCALITY: Canada; Wainright, Alberta, January 28, 1923; S. Hadwen, collector.

TYPE: United States National Museum, Helminthological Collections, No. 26101; paratypes Nos. 25960, 26103.

This species is most closely related to *O. trifurcata* Rans., and in his key (Ransom)[3] it would run out at that species. The spicules are, however, a trifle smaller in the present species, and are of a different conformation.

[3] RANSOM, B. H. THE NEMATODES PARASITIC IN THE ALIMENTARY TRACT OF CATTLE, SHEEP, AND OTHER RUMINANTS. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 127, 132 p., illus. 1911.

NEMATODES FROM BEAVER

Travassosius americanus, n. sp.

MALE: 11 mm. long, slender, 96 μ in diameter at the level of the esophage-intestinal valve, diameter increases evenly to 116 μ at a point just in front of the prebursal papillae. Head small, 30 μ in diameter, buccal capsule absent, esophagus 575 μ long; its greatest diameter is 65 μ near the posterior end. Nerve ring surrounds esophagus at a plane 260 μ behind the anterior end. The excretory pore is about 345 μ from the head. The cervical papillae are large, conical, and prominent, about 50 μ behind the excretory pore. The prebursal papillae are lateral, 50 μ in front of the anterior margin of the bursa. The bursa is in most respects similar to that of *T. rufus* Khalil.[4] The most conspicuous difference is in the formation of the dorsal ray, which bifurcates at a point 56 μ from the base of the ray and produces two branches, each 16 μ neither of which bifurcates at the extremity. The spicules are two, and similar, from 148 to 152 μ long. Gubernaculum is absent.

FEMALE: 12 mm. long; diameter of head 30 μ, greatest diameter 128 μ, near vulva. Esophagus about 600 μ long, nerve ring 280 μ behind its anterior end. Excretory pore 430 μ, cervical papillae 460 μ from anterior end. Vulva about 3 mm. from the tip of the tail. Tail slender, slightly recurved at its tip, anus 240 μ from the tip. The length of the combined portions of the ovejectors, including the sphincter muscles is about 630 μ; each sphincter alone measures 45 μ long by 60 μ in diameter. The eggs are oval, thin shelled, from 82 to 90 μ in length by 43 to 50 μ in width.

HABITAT: In stomach of *Castor canadensis,* National Zoological Park, Washington, D. C., January 23, 1924; E. A. Chapin collector.

TYPE: United States National Museum, Helminthological Collections, No. 25966, paratypes No. 26107. Paratypes also deposited in the British Museum (Natural History).

This species is very closely related to *T. rufus* Khalil, the type of the genus. The spicules are smaller in the present species and are somewhat different in conformation.

Castorstrongylus n. gen.

Strongylidae, Strongylinae, Cylicostomeae. Medium-sized worms, sexes of approximately the same size. Buccal capsule large, heavily walled, without teeth at base. External corona of fimbriated elements, internal corona of short, stout peglike elements. Cervical papillae and excretory pore a short distance apart, both behind the nerve ring. Bursa of male moderate. Ventral rays close together and parallel, externo-dorsal arising free of the trunk of the dorsal, dorsal twice bifurcated. Vulva near anus, uteri directed anteriorly, convergent.

HABITAT: In colon of a rodent.

TYPE AND ONLY KNOWN SPECIES: *Castorstrongylus castoris* n. sp.

The type species has the habitus of a true Strongylus while the bursal and other characters usually chosen as of tribal importance place it in the neighborhood of the cylicostomes.

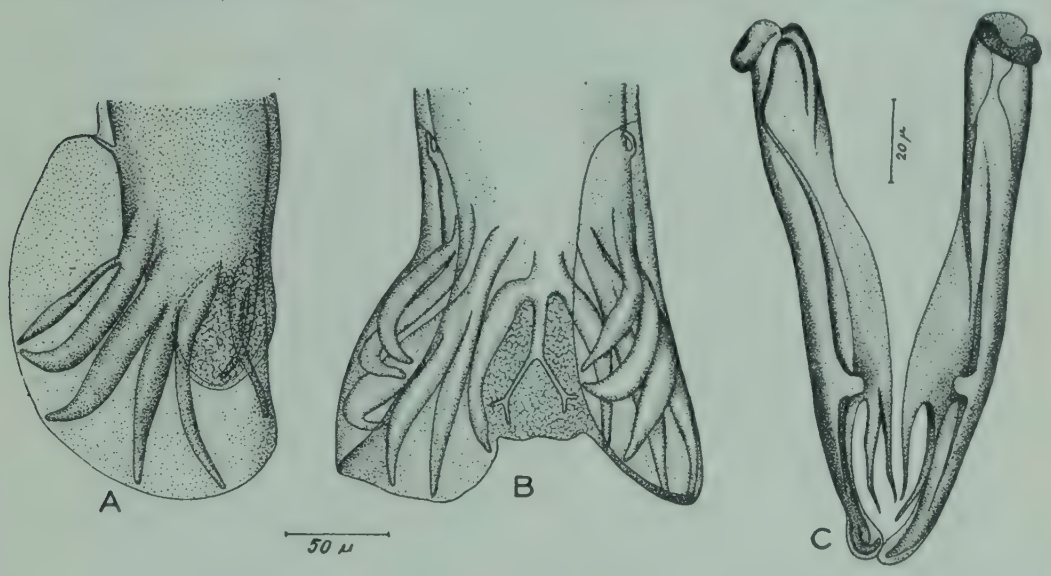

FIG. 2.—*Ostertagia bisonis,* n. sp. *A,* bursa of male from side; *B,* same, ventral view; *C,* spicules

[4] KHALIL, M. TRAVASSOSIUS RUFUS, GEN. ET SP. N.: A NEMATODE (TRICHOSTRONGYLIDAE) PARASITIC IN THE STOMACH OF THE NORWEGIAN BEAVER, Ann. and Mag. Nat. Hist., 10: 281–289, illus. 1922.

Castorstrongylus castoris, n. sp.

SYNONYMS: *Sclerostoma* sp. Morgan, 1868;[5] *Strongylus* sp. Hall, 1916.[6]

MALE: 11 mm. in length; greatest diameter 645 μ, at about the middle of the length. Mouth opening anterior, surrounded by a prominent collar 285 μ in diameter by 60 μ in height. Circumoral papillae situated on collar, submedian papillae prominent, lateral papillae obsolete, amphids distinct. Buccal capsule large, walls 25 μ thick, internal diameter (dorso-ventral) 240 μ, depth 195 μ, dorsal gutter distinct, toward base of capsule lying in a thickened ridge. External corona composed of 24 elements, each element fimbriated at its anterior border, num-

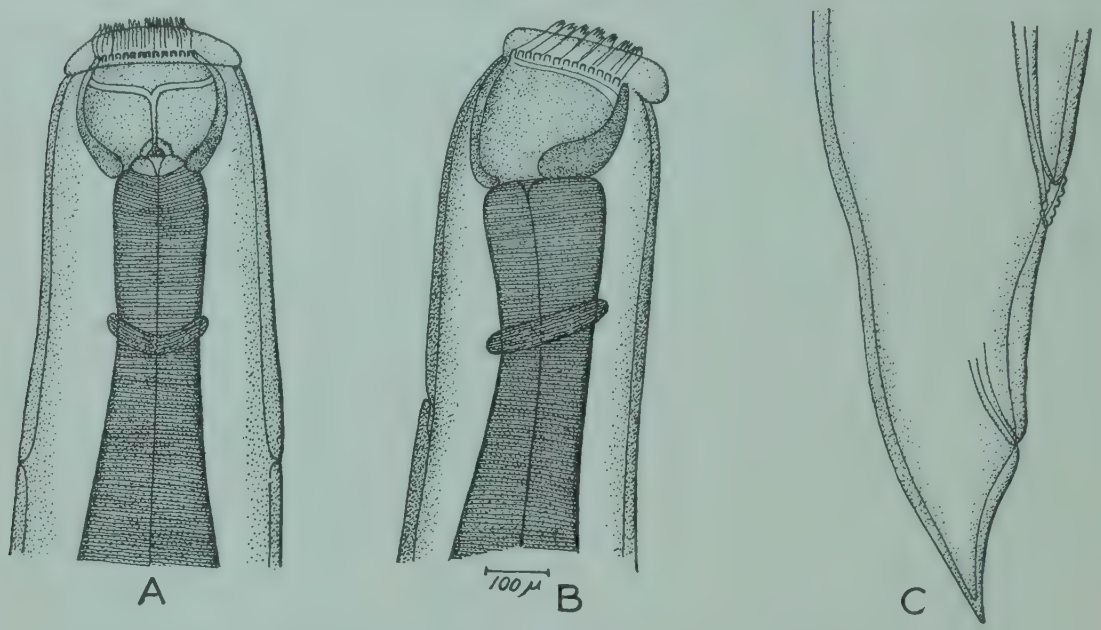

FIG. 3.—*Castorstrongylus castoris,* n. sp. *A,* anterior end of male, ventral view; *B,* same, lateral view, *C,* posterior end of female

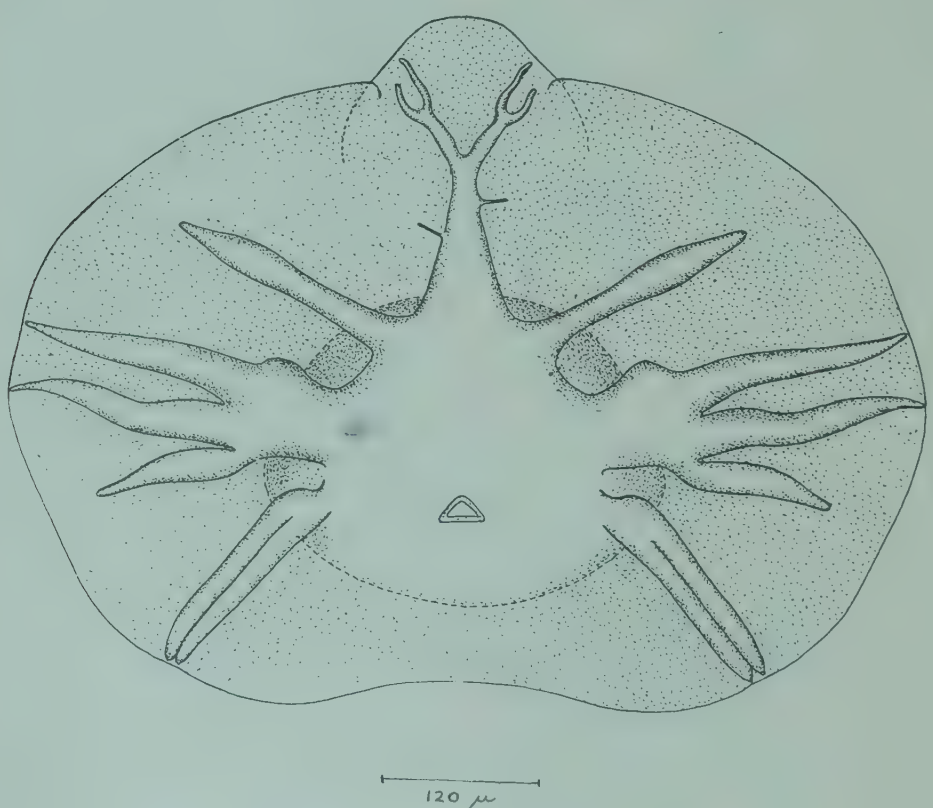

FIG. 4.—*Castorstrongylus castoris,* n. sp. Bursa, male

[5] MORGAN, L. H. THE AMERICAN BEAVER AND HIS WORKS. 330 p., illus. Philadelphia. 1868.
[6] HALL, M. C. NEMATODE PARASITES OF MAMMALS OF THE ORDERS RODENTIA, LAGOMORPHA, AND HYRACOIDEA. Proc. U. S. Nat. Mus. 50: 1-258, illus. 1916.

ber of divisions varying from 5 to 7. Internal corona composed of 48 peglike elements, each peg about 8 μ long, cylindrical, rounded at apex. Internal corona situated within the collar, about 30 μ behind the anterior margin. Esophagus 1.2 mm. long, clavate, greatest diameter 250 μ near the intestinal valve, diameter at buccal capsule 195 μ, diameter at nerve ring 165 μ. Nerve ring surrounding esophagus at its anterior fourth. Cervical papillae small, situated just anterior to the middle of the esophagus, excretory pore just behind cervical papillae. Bursa transversely oval, dorsal lobe trapezoidal, dorsal trunk bifurcated, at about half its length, each fork bifurcated near tip. On each side of main trunk there is an exceedingly short and slender branch. Externo-dorsal ray moderately thick, parallel, originating halfway between the main trunks of the dorsal and lateral rays, ending some distance from the margin of the bursa. Lateral rays stout, postero-lateral and medio-lateral; not close together but subparallel; tips at margin of bursa, end of externo-lateral remote from the bursal margin; ventral rays similar in size to the laterals, close together, parallel, ending on bursal margin. Spicules 1.4 to 1.5 mm. long, slender, somewhat twisted. Gubernaculum wanting (fig. 4).

FEMALE: 12 mm. in length, greatest diameter 645 μ at about middle of length. Mouth collar slightly lower that that of male but of the same diameter. Dorso-ventral diameter of buccal capsule 240 μ, equal to the greatest depth. External and internal coronae similar to those of male. Esophagus 1.10 mm. long, 300 μ in diameter just before the intestinal valve, nerve ring at anterior fourth of esophagus, excretory pore at the level of the nerve ring, cervical papillae 255 μ behind the excretory pore. Tail conical, slender; anus 315 μ in front of extreme tip, vulva 375 μ in front of anus. Eggs 83 to 92 μ long by 51 to 54 μ wide, up to the eight-celled stage in uterus.

HABITAT: In colon of *Castor canadensis*, National Zoological Park, Washington, D. C.; January 22, 1924; E. A. Chapin, collector.

TYPE: United States National Museum Helminthological Collections, No. 25967; paratypes No. 26104. Paratypes also deposited in the British Museum (Natural History).

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FURTHER INVESTIGATIONS OF INFECTIOUS EQUINE ANEMIA IN NEVADA[1]

By LEWIS H. WRIGHT

Nevada Agricultural Experiment Station[2]

INTRODUCTION

Infectious equine anemia was first reported in Nevada by Mack (*5*, *6*, *7*, *8*), in 1909; (*9*)[3] in 1909. He made extensive observations on the pathology, bacteriology, and symptomatology of the disease, but with one possible exception was unable to transmit it to other horses, and so failed to arrive at any positive conclusion as to its etiology. All of Mack's material came from one portion of the State, the region of the south fork of the Humboldt River in Elko County, where the disease had been prevalent and had caused extensive losses periodically for a number of years.

Since this first report the disease has been found and studied around Fallon in Churchill County, near Washoe in Washoe County, and along the north fork of the Humboldt River. There have been reports of its having occurred around Yerington, Pahrump, Lovelock, and Battle Mountain, but these reports have not been confirmed.

It is especially interesting to note that all of the infected areas are a considerable distance from one another, and, as far as can be ascertained, there has been little or no interchange of animals among them. In fact, many of the infected animals had never left the ranch where they were raised.

From 1909 until 1918 the disease was apparently quiescent and caused very little loss. In fact, material for experimental purposes was at times unprocurable and very little work was done during this period. In September, 1917, material was procured from Antelope Valley and some experiments were undertaken. This outbreak subsided, however, and the experimental virus was lost, owing to its lack of pathogenicity.

In September, 1918, material was obtained from natural field cases at Fallon. Most of this report is based on experimental work done with virus from this source.

In this report it is intended to touch some phases of the disease not solved by Mack, such as transmissibility, differential diagnosis, etc. Mack's (*6*, *7*, *8*, *9*) observations on the pathology and symptomatology of the disease are sufficiently complete to need little repetition here. If the form of anemia with which Mack worked is identical with that reported from other States and foreign countries, the thing that stands out conspicuously in his experiments is the great difficulty which he encountered in transmitting the disease. This may have been due to the fact that he inoculated only animals in districts where the disease was prevalent. In other words, he may have used no really susceptible animals. His fear of introducing the disease in new areas caused him to refrain from inoculation experiments at Reno.

If the disease upon which the present investigations are based is the form of anemia usually found, this work will present very little that is wholly new. However, this report may establish some new facts concerning the local disease, especially its transmissibility, and may add others to show that it is the same disease encountered elsewhere.

EXPERIMENTAL DATA

The observations of the writer agree with those of Mack (*6*) in this, that there are three types of the disease, namely, acute, subacute, and chronic. Although distinct, the three types are separated by no hard and fast line, and a case that is thought to be acute may develop into subacute or chronic, or vice versa. Ranchers have reported some acute fatal cases, but there is considerable evidence to support the belief that the cause of death in these cases was not infectious anemia. No opportunity was offered to check up on these cases by animal inoculation.

In these later observations the blood examinations were made in the chronic field cases to exclude intestinal para-

[1] Received for publication June 11, 1924; issued June, 1925.

[2] Resigned June 1, 1920.

[3] Reference is made by number (italic) to "Literature cited," p. 692.

sites, the differentiation being based partly on the presence or absence of an eosinophilia. This method proved sufficiently satisfactory until a group of eight colts, 2 to 4 years of age, was encountered. These colts had been in and near places where infectious equine anemia was known to be present. Physical examination showed the usual symptoms of this disease, which are similar to strongylosis, some of the animals running a temperature of 105° F. or more. The high temperature and the absence of an eosinophilia pointed very strongly to infectious equine anemia. The disease was diagnosed as such, and since the animal had been ailing for some time, was considered of the chronic type.

The animals were brought to the laboratory for further study and treatment. Repeated blood examinations over a period of weeks demonstrated the usual number of eosinophiles and there was no appreciable lymphocytosis, the differential count being well within the usual boundaries for normal animals. A pronounced anemia was noted in some of the colts, the red cells being as low as 3,888,000 per c. mm. Eventually, all the animals died or were killed when death was imminent.

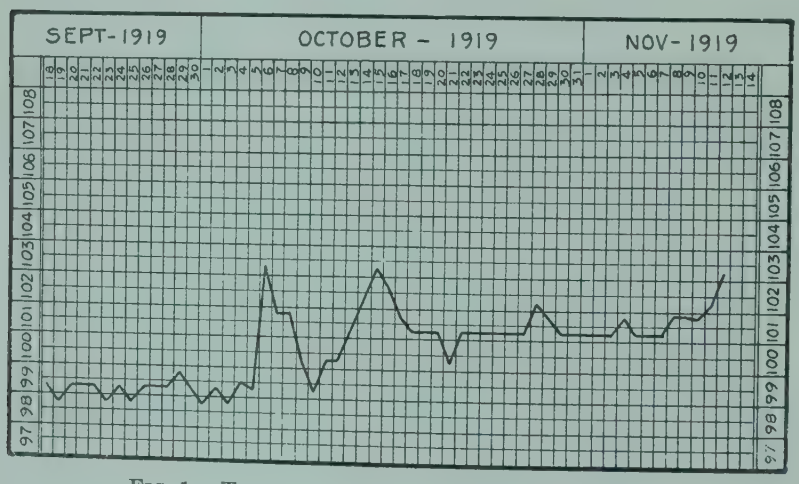

FIG. 1.—Temperature record of animal No. 2965

With one exception, all showed extreme infestations with Strongylus and Cylicostomata; this one exception having 1,000 Gastrophillus in the pylorus. Repeated blood inoculations on many susceptible animals failed to reproduce the disease and for this reason infectious equine anemia can positively be excluded. In this connection it might be said that in one horse which consistently showed from 15 to 17 per cent of eosinophiles, but few parasites of any kind were present at autopsy. It can safely be stated that the presence or absence of an eosinophilia is of little or no aid in making a diagnosis of infectious equine anemia, the only positive diagnosis possible at present being that secured by reproduction of the disease in an animal by inoculation.

For the inoculation great care was taken to use only healthy animals and to see that there was no chance of accidental infection.

No. 2965.—On September 18, black gelding 12 years of age, in good flesh and spirits, weighting 1,185 lbs., was given intravenously 480 c. c. of whole blood from No. 2597, a chronic experimental case produced by Berkefeld-filtered blood from a typical field case; September 25, given intravenously 150 c. c. of whole blood from No. 2597; October 9, given intravenously 200 c. c. of whole blood from 2597; November 1, weight, 1,055 lbs.; November 7, weight 990 lbs.; edema of abdomen; very weak but appetite very good; November 13, unable to stand; continued loss of weight; November 14, animal died. During the illness this animal showed the typical symptoms of infectious equine anemia. Blood examination on November 1 showed a slight decrease in red cells as compared with a count made on the day of the first inoculation. Figure 1 gives the temperature record of this animal.

No. 2563.—On October 22, middle-aged gelding in good physical condition weighing 940 lbs., was given subcutaneously 100 c. c. of defibrinated blood from No. 2250, a typical acute field case. The first symptoms of the disease were shown on November 5. The animal gradually lost weight, became weak, the mucous membranes of the eyes and the nose took on a yellowish-red tinge and on November 13 its weight was 840 lbs. On November 14 there was some hemorrhage from the right nostril. On November 15 the animal was unable to get up, there was some edema of the legs, and hemorrhage beneath the nasal mucosa. The animal died November 17. There had been a gradual fall in the number of red cells and in the percentage of hemoglobin (fig. 2).

No. 2684.—On March 6, healthy gelding 8 years of age, weighing 1,015 lbs., was given, partly subcutanously and partly intravenously, 350 c. c. of a solution made by taking 250 c. c. of whole clotted blood from No. 2561, a typical field case, and shaking it with physiological salt solution and straining out the clumps of fibrin. The first symptoms were noted on March 18 when the animal appeared dejected and listless. He gradually grew worse and on April 6 there was edema of the abdomen and prepuce, and the mucous membranes of the eyes and nose were reddish yellow. On April 11 the weight was 910 lbs. On April 15 he was very weak, dejected, and passed bloody urine. Death occurred on April 16. The hemoglobin and red

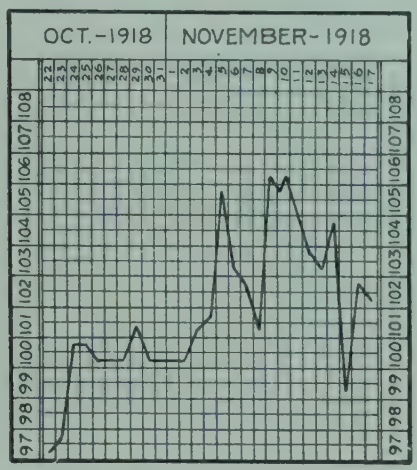

FIG. 2.—Temperature record of animal No. 2563

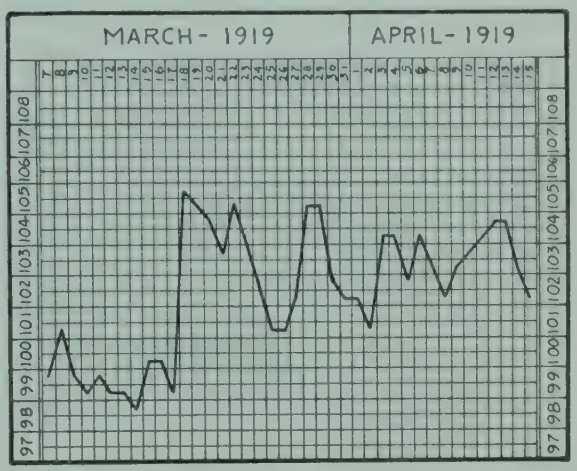

FIG. 3.—Temperature record of animal No. 2684

cells gradually decreased and the lymphocytes slowly increased during the illness of the animal (fig. 3).

No. 2932.—On August 25, aged healthy gelding, weighing 1,195 lbs., was given 50 c. c. of whole blood, intravenously, from experimental case No. 2796. On September 12, 200 c. c., and on September 25, 400 c. c. more were given. The first symptoms with edema were noticed on October 6. The animal gradually became worse with loss of flesh and spirits and continued ill until early in January, when he apparently recovered and remained well until he was killed on February 17, being apparently healthy at this time. On October 9 the hemoglobin was 60 per cent, the red cells 5,834,000 and the lymphocytes 42.2 per cent. This condition gradually improved and was normal at the death of the animal (fig. 4).

No. 2283.—On August 21, a middle-aged healthy gelding weighing 1,070 lbs., was given intravenously 30 c. c. of defibrinated blood from a typical subacute case. The first symptoms—weakness, loss of flesh and spirits, edema of the prepuce and pale yellowish mucous membranes—were noticed in about six weeks. This condition gradually became worse with development of edema of the legs and abdomen, nasal hemorrhage and polyuria. The blood drawn from the veins was thin, did not clot readily, and the corpuscular volume was about one-fifth the total volume. The serum was tinged with yellow. The general condition of the animal gradually improved and he was in fairly good form during the latter part of October. Some severe exercise at this time, however, was apparently too much for his weakened resistance; he rapidly became worse and died on November 4. At the time of death the hemoglobin was 40 per cent and the red cells 3,936,000 (fig. 5).

No. 2801.—On June 9, a middle-aged healthy mare, weighing 850 lbs., was given intravenously 450 c. c. of whole blood from No. 2597. On June 6 and July 3, respectively, 400 c. c. of blood were injected. On August 13 and September 12, respectively, 500 c. c. of blood were injected. The first symptoms were noticed on September 13, a brownish-red nasal discharge, weakness, and general indisposition. The animal gradually became worse and died on September 29. The hemoglobin and red cells decreased slightly during the illness of the animal (fig. 6).

No. 2586.—On November 12, a healthy mare 10 years of age, weighing 939 lbs., was given intravenously 60 c. c. of mixed defibrinated blood from Nos. 2316, 2317, and 2318, three suspected field cases. On November 25, she was given 30 c. c. more of defibrinated blood from No. 2317 and No. 2318. The first symptoms were noticed two weeks after this last injection, and the animal died on December 12 (fig. 7).

No. 2796.—On June 4, a healthy gelding 10 years of age, weighing 1,085 lbs., was given intravenously 800 c. c.

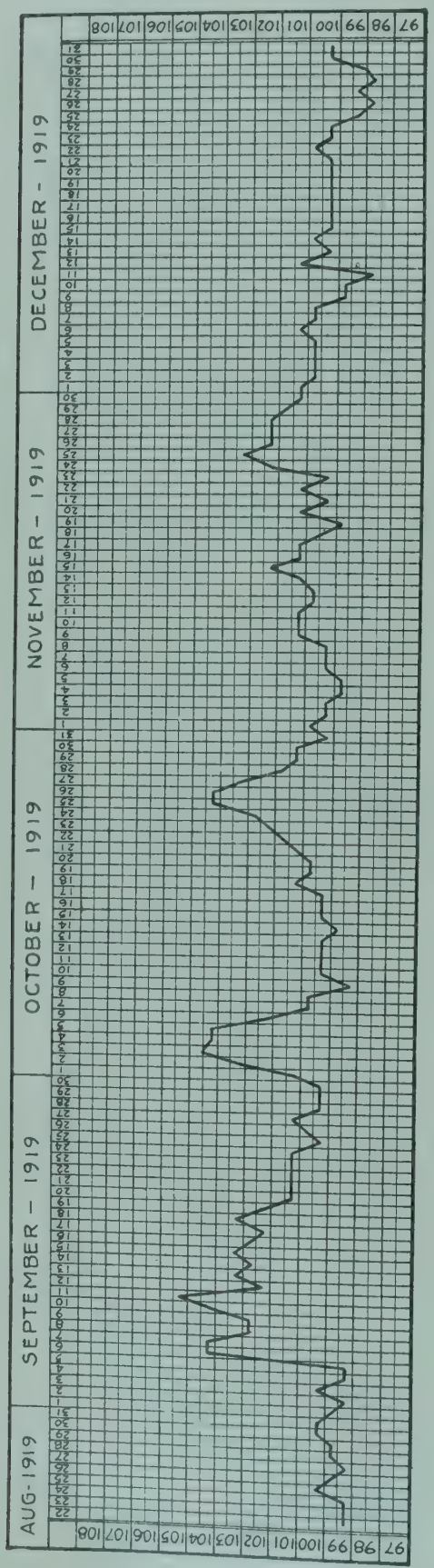

FIG. 4.—Temperature record of animal No. 2932

of blood serum from No. 2913, a typical field case. On June 19 this animal was given 500 c. c. more of serum from the same field case. The first symptoms of disease were noticed on June 24. The animal slowly grew worse until about August 15 when he became very ill and remained so until September 1. He gradually improved and remained well from about September 12 until February 19, when he was killed. During the animal's illness the red cells decreased, the hemoglobin was below normal and the lymphocytes were above normal. Later they came back to normal and remained so until the animal was killed. Figure 8 shows the temperature record of this horse. From June 4 until July 27, and from September 30 until he was destroyed, the temperature was normal.

No. 2676.—On February 19, a healthy gelding 12 years of age was given intravenously 750 c. c. of whole blood from No. 2597, an experimental case produced from a typical field case by Berkefeld filtrate. The first symptoms were noted on February 27. Later the mucous membranes became pale and tinged with yellow and there was some serous nasal discharge. The condition of the animal gradually became worse and he died on March 23. The red cells and hemoglobin showed about a 25 per cent reduction during the illness of the animal (fig. 9).

Since the disease was found to be transmissible to susceptible animals by inoculation of whole and defibrinated blood, it was desired to see if it could be transmitted by the injection of Berkefeld-filtered material. Accordingly, blood was drawn from some of the field and experimental cases, Berkefeld-filtered, and injected into susceptible animals. Before use the filters were tested with known cultures of bacteria to check their efficiency.

No. 2008.—On November 2 a healthy mare 12 years of age, weighing 925 lbs., was given intravenously 10 c. c. of Berkefeld-filtered blood serum from experimental case No. 1948. This animal remained healthy until May 24, when many petechiae were noted in the nose, with some hemorrhage. On June 3 she was definitely ill. She gradually became worse, however, developing reddish-yellow membranes, serous nasal discharge, edema of the legs and abdomen, and loss of weight, weighing 748 lbs. She died on July 14. Very little change was noted on blood examination until the late stages of the disease, when there was about a 20 per cent reduction in the hemoglobin and red cells (fig. 10).

No. 3036.—On January 28 a healthy mare 14 years of age was given intra-

venously 100 c. c. of Berkefeld-filtered blood serum from No. 2597, an experimental case produced with filtrate. The first symptoms were noted on February 20. The progress of the disease was very rapid, and the animal died on February 22 (fig. 11).

No. 2597.—On November 10 a healthy gelding 2 years of age, weighing 650 lbs., was given 30 c. c. of Berkefeld-filtrate from experimental case No. 2563. On November 20 he was given intravenously 30 c. c. of filtrate of blood and splenic emulsion and 40 c. c. more on the following day. On January 16 he was quite ill and remained so for some time, gradually recovering and being apparently well by February 15, when he weighed 732 lbs. About March 18 he had a relapse and was very ill for a week. His weight fell to 573 lbs. At this time he was so ill that he was expected to die, but he gradually recovered and

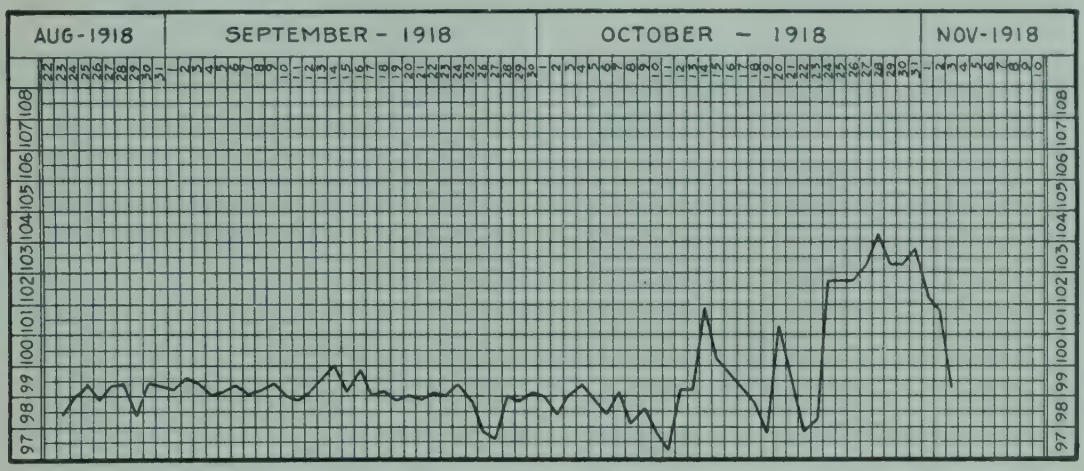

FIG. 5.—Temperature record of animal No. 2283

was apparently well by the middle of April. From this time until he was killed on February 26, nearly a year later, he showed no departure from the normal. During this time, however, he was an active carrier of the virus, many subinoculations being made from him, some of which are reported here. Figure 12 shows his temperature record. During the break in his temperature record, and from April 11 until his death, the temperature was normal. During the illness of this animal the hemoglobin and red cells were decreased about 20 per cent and the lymphocytes went as high as 54.4 per cent.

As a check on the diagnosis, and in order to eliminate acute bacterial diseases, blood was taken at nearly every autopsy for subcutaneous and intraperitoneal inoculations on rabbits and guinea pigs. From time to time emulsions from the liver, spleen, and lymph glands were also injected. At no time was there any evidence of a reaction in one of these animals.

Attempts were made to reproduce the disease in animals other than horses, but all of these failed. Common house mice, rabbits, guinea pigs, calves, sheep, and pigs were injected with blood and kept under careful observation for periods varying from weeks to several months, all with negative results.

DISCUSSION

This report can add little to the autopsy findings reported by Mack (*6*), with the exception of those in the long bones. Many writers have considered that the findings in the bone marrow are characteristic of the disease. In a previous paper the writer (*18*) has quite conclusively shown that these changes are not characteristic and may even be greater in other diseases than in infectious equine anemia.

At all autopsies cultures were made on neutral plain agar and neutral plain broth. At autopsies and at times during the acute illness of the various cases cultures were made on special media. These media included normal rabbit blood agar; 10 per cent horse serum, 2 per cent agar and physiological saline; 15 per cent horse serum and water; 5 per cent horse serum and water; 15 per cent horse serum and physiological saline; 5 per cent horse serum and physiological saline; 25 per cent horse serum and water; 12 per cent horse serum and water; 25 per cent horse serum and physiological saline; 12 per cent horse serum and physiological saline. Some of these cultures were oiled in order to secure anerobic conditions, a piece of rabbit testicle being added in some cases and a piece of rabbit kidney in others. All cultures were incubated

at 37.5° C. for varying periods of time, some as long as two months. At no time was there any growth that could be considered anything but contamination from outside sources. These results do not agree with those of Miyagawa, Taniguchi, Nagao, and

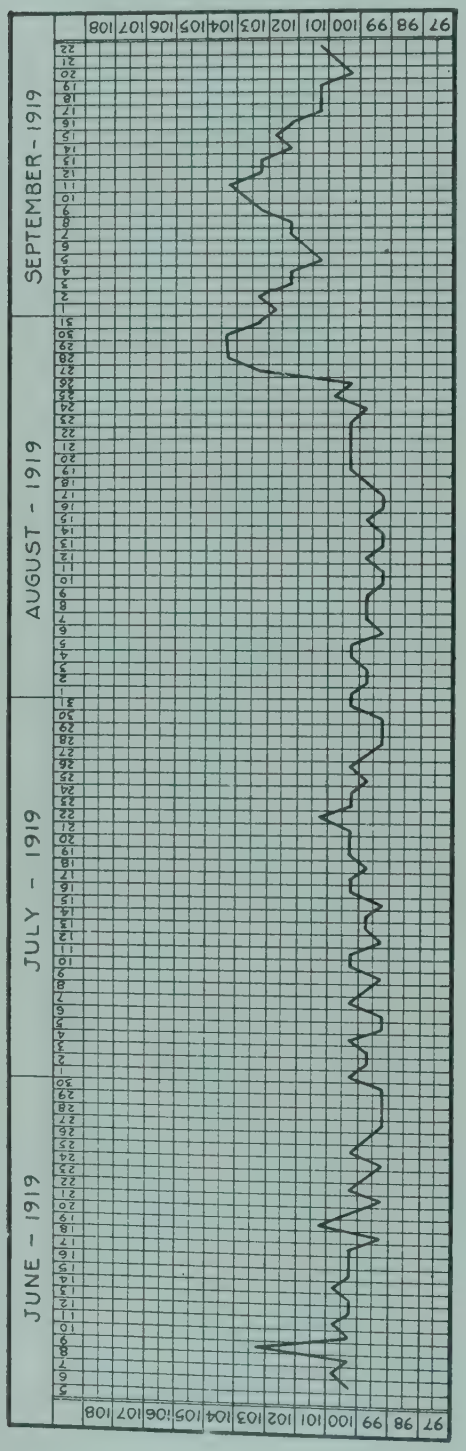

FIG. 6.—Temperature record of animal No. 2801

Takemoto (*11*), who were working with what is supposed to be the same disease in Japan. These investigators claim that spirochaetes can be observed in direct smears from the blood stained with Giemsa's stain. Almost without exception the blood smears made by the writer have been so stained, but at no time has anything resembling a spirochaete been seen in them. This statement is based on hundreds of examinations made over a period of years. At various times fluids from the edematous areas also was smeared and stained for spirochaetes by the silver and India-ink methods, but the results obtained were negative.

One thing that stands out conspicuously in the severest cases of anemia is the lack of erythroblasts. These are seldom encountered and then only in small numbers. Apparently the regenerative powers of the animals are unable to cope with the loss of red blood cells. Perhaps this lack of regeneration or red cells is one of the causes of the acuteness of the disease, although the chronic cases are the ones that show the most pronounced anemia, the acute cases in many instances showing little or no loss of red cells.

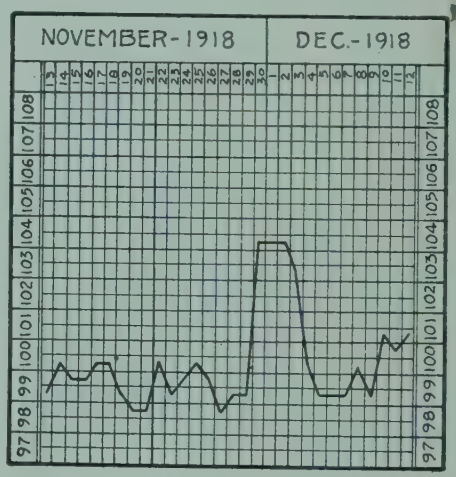

FIG. 7 —Temperature record of animal No. 2586

This lack of ability to regenerate red cells may be one of the reasons why there are no marked changes in the bone marrow. One would not expect to find extensive marrow changes unless there was active blood regeneration.

There seems to be a greater loss of red cells in the field cases than in the experimental ones. This may be accounted for by the overwhelming quantities of blood injected into the latter and the consequent acuteness of the disease produced. The field cases as a whole are apparently less acute than the experimental ones.

During the routine examination of the stained smears there have been observed the so-called intracorpuscular bodies, described by Mack (*10*). What relation these have to the disease in question is as yet unsettled. In these examinations they have been found present in many cases. They have also been found in normal horses

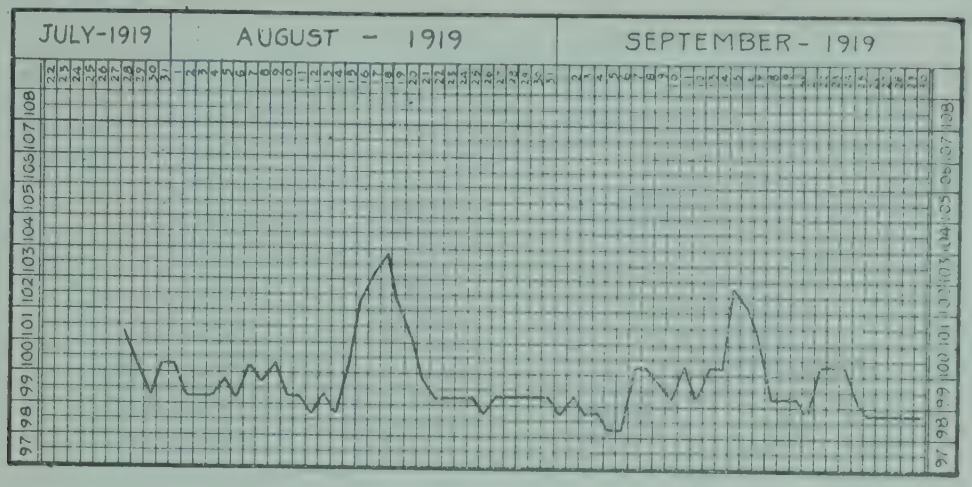

FIG. 8.—Temperature record of animal No. 2796

and in horses affected with other diseases. They are especially numerous in the blood of horses used for the production of hemorrhagic septicemia antiserum. Later developments may show that they are peculiar to this disease or that they are some form of antibody or immune body. Their presence in greater numbers in the blood of the hyperimmune horses would tend to confirm this. In this connection further observations will be of value.

DIAGNOSIS

Too much emphasis can not be laid on the fact that the only positive differential diagnosis of infectious anemia is by the reproduction of it in a susceptible animal. Sometimes, however, a fairly accurate diagnosis can be made from a physical examination, including a blood count. If the case can be observed for a few days in order to get a temperature curve this is also a great help. From the writer's observations the two conditions most likely to be confused with the disease are parasitisms and nonspecific secondary anemias. The differentiation from parasitisms has been mentioned previously. That of nonspecific anemias requires considerable care and judgment but is apparently less difficult, for as a rule, some contributing factor can be found.

Usually the autopsy lesions are of some help in diagnosing the disease. In general, there is an enlargement of the spleen, a peculiar brownish, granular condition of the liver, the latter being very friable, and in many cases there are extensive epicardial and endocardial hemorrhages.

TRANSMISSION

Little work has been done here on the methods by which the disease is spread. Carré and Vallée (*1, 2, 3, 4*) and others believe that it is spread by urine, feces, and other body discharges, contaminating the food and drink of healthy horses. Scott (*12, 13, 14, 15, 16, 17*) has apparently proved that flies spread the disease. From the investigations made by the writer it seems highly probable that flies are an important factor in its dissemination. When material was available, a healthy horse was put with the sick; they ate from the same manger, drank from the same tub, and were kept in intimate association with one another. This experiment was repeated several times,

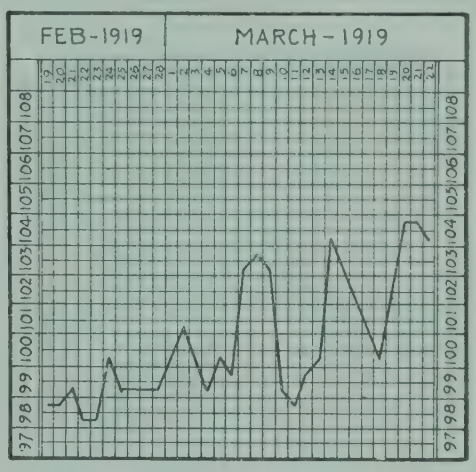

FIG. 9.—Temperature record of animal No. 2676

so that there is little chance that an animal which was naturally resistant would be used in every instance. One horse was kept for over 16 months in continual contact with the sick ones. None of the experimental animals showed any evidence of having contracted the disease. It is of interest to note that there were few, if any, biting flies present where these animals were kept.

One should not overlook the important fact that the disease is most prevalent in the season of the year when biting and sucking flies are most abundant, namely, the late summer and fall. If one can take a small quantity

of infected blood in a syringe and transfer it to an animal and thus reproduce the disease it seems highly probable that a biting fly can achieve the same result. Occasionally droplets of blood have been seen on the skin of a horse, caused by the bite of a fly. There is apparently some relation also between the number of cases of the disease in a given year and the number of flies, the more flies there are the more cases appear.

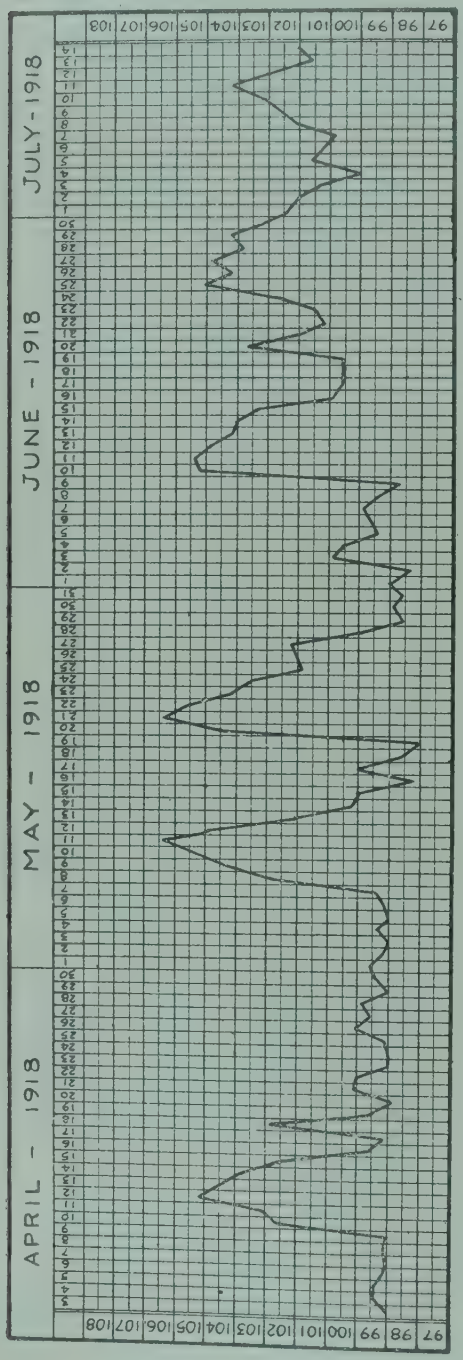

FIG. 10.—Temperature record of animal No. 2008

CONTROL

No satisfactory treatment for the disease is known. Many investigators have tried various methods with no uniform success. Some have recommended the use of such drugs as sodium cacodylate, atoxyl, trypan-blue, trypan-red, quinine, tartar emetic and various other compounds. Under critical observations, however, all of these have failed. Nearly all of the known methods of treatment have been tried by the writer both in the field and in experimental cases. At no time has anything resembling a cure been found. Some known cases have lived for some time and been apparently well, but inoculations from them have shown that they were still carriers of the virus. In the light of our present knowledge of the disease, there might even be danger in attempting to cure it, since cases supposedly cured might become sources of infection to other animals. What is probably necessary is the immediate distruction of all suspected cases. Definitely diagnosing the disease by animal inoculation is a long and expensive process and is practically out of the question in routine work. The control of biting flies, clean sanitary premises, and good hygienic surroundings for the horses are probably the greatest factors in the control of the disease.

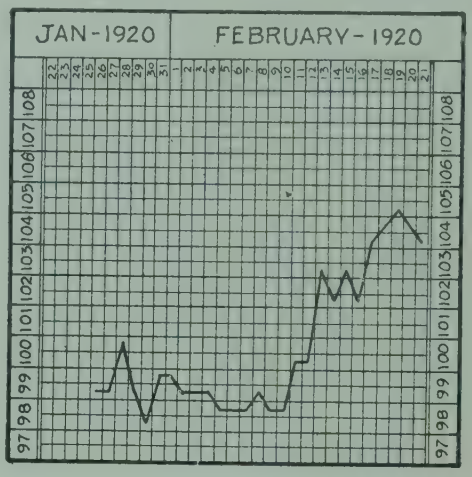

FIG. 11.—Temperature record of animal No. 3036

CONCLUSIONS

There exists in Nevada a disease among horses which is apparently identical with that known in other States and countries as infectious equine anemia, swamp fever, infectious anemia, etc. The disease is characterized clinically by irregularly remittant fever, rapid emaciation, marked loss of energy, depletion of red blood cells in most cases, edema, usually bloody nasal discharge, and eventually death. The mortality is nearly 100 per cent, real recovery rarely, if ever, taking place. The disease is transmissible to other horses by the injection of infected blood or splenic emulsion, the period of incubation being from about two weeks to several

months. The etiological factor is apparently ultramicroscopic, since the disease can be reproduced by the injection of Berkefeld filtrates, and is not recognizable by ordinary staining methods in smears from the blood, tissues or exudates and transudates. The natural mode of transmission is not known, but is most likely through the bites of insects. Methods of treatment thus far used have proved unsuccessful, killing the infected animals being necessary to prevent spread of the disease. A positive diagnosis can only be secured by animal inoculation. Animals other than equines are not susceptible to the disease.

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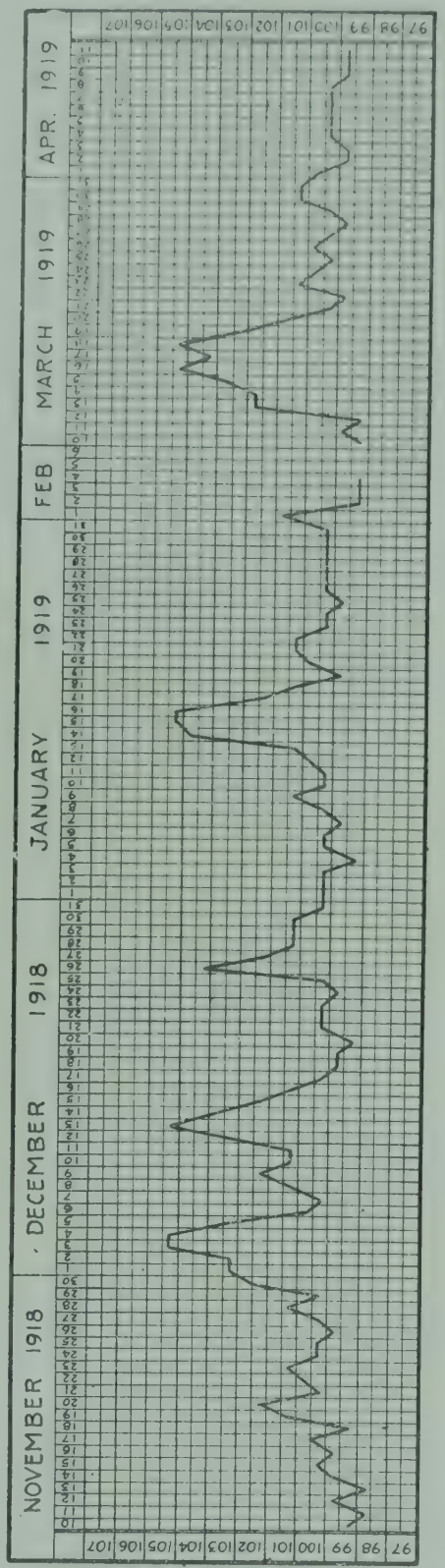

FIG. 12.—Temperature record of animal No. 2597

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THE USE OF LIABILITY RATINGS IN PLANNING FOREST FIRE PROTECTION[1]

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INTRODUCTION

Two main objects were in view in undertaking this study. In the first place, it was desired to ascertain if some scientific method could be found by means of which it would be possible to determine how much money can justifiably be spent for fire protection on the national forests. The second object was to provide a basis for the proper distribution of available protection funds between the different units of the organization.

The results of the study seem to indicate that so far, with the inadequate data available, no absolute mathematical rules or formulae can be established to fulfill either of these purposes. Scientifically accurate formulae require accurate basic data, which can be gained only through years of intensive research, and through detailed records, carefully kept for a considerable period. Moreover, the correct application of such formulae would require accurate detailed knowledge of the resources that we want to protect, which can be gotten only by means of intensive survey and mangement plans for the entire national forest area.

It is believed, however, that the study, even though based on admittedly unsatisfactory data, has yielded some material which, supplemented by a fire plan reconnaissance, will be of considerable value both in helping to determine the total amount of expenditures justifiable, and in distributing allotments within the organization. As better data accumulate, upon which to base more reliable figures than those worked out in the following pages, they will become more useful for these purposes. It is extremely important that such data be collected and kept as permanent records so that they may be utilized as the basis for future research.

BASIC PRINCIPLE GOVERNING EXPENDITURES

The best measure by which to judge the sufficiency of any fire protection organization is the net result accomplished. This net result may be expressed in terms of cost of protection plus losses incurred in spite of protection; and the smaller this sum, the more efficient the protection. As one factor, cost of protection, goes up when the other, loss, goes down, it is evident that there will always be some point below which the sum can not be reduced. Up to this point, expenditures for protection are justifiable.

Since the object in view is to reduce the sum of cost plus loss to a minimum, and not to eliminate all loss, regardless of cost, it is evident that justifiable costs should be determined by weighing against them the losses likely to be incurred.

Protection costs are in two distinct categories. One, which may be called primary protection, includes the cost of the organization for prevention, detection, and suppression (including personnel, equipment, and improvements), and is determined in advance. The second includes actual costs of suppression, such as temporary labor, subsistence, and transportation, as well as the time of forest officers taken off from other work. These costs, like losses, can not be determined in advance, but together with the losses depend upon the occurrence of fires. They can not, or should not, be limited by the arbitrary allotment of funds in advance, because with exceedingly few exceptions all fires must be fought, the question of "how soon" being answered by weighing probable losses plus suppression costs against the expenditures required to attack them within given periods. Even in the case of open lands with low liability, it

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will usually be necessary to suppress fires to keep them from running over on lands with greater liability, or because of the consequent effect on the fire hazard in general.

The principle may be illustrated by a diagram (fig. 1), which shows the curve for loss plus suppression costs (X-Y) descending as the line representing primary protection costs (A-B) rises, while the curve S-T, representing the sum of the two, falls to a point P, then rises steadily. The expenditure at which P is attained, or E, represents the proper amount to spend for primary protection. A greater expenditure might indeed reduce the loss and suppression cost, but not sufficiently to reduce the total, and so might not be justified.

The purpose of this study, then, was to determine whether it is possible to rate the liability of loss as well as the probable cost of suppression, which together may be termed the total liability. No attempt was made to actually rate the fire hazard and liability for specific forest units, but principles and methods have been worked out as a basis for a detailed field survey or "fire reconnaissance," which must necessarily lie at the foundation of any rating for specific forest units.

FACTORS OF HAZARD AND LIABILITY

The probability of loss is governed primarily by the values of destructible resources, and by the hazard, or chance of their destruction as a result of exposure to fire. Values of forest resources may be classified under the following heads: (1) Timber, including mature timber, young growth, and the forest capital, which includes soil productivity; (2) forage; (3) indirect values, including watershed protection (regulation of streamflow and prevention of erosion and floods) and occupancy values, such as recreational use, improvements, game resources, and the like.

FIRE HAZARD

The chance of destruction by fire of the values on a given forest area depends upon the probability of its being burned over, and upon the probability that the values will be destroyed as a result of such burning. Its chance of being burned over depends upon whether fires will start on or near it, and upon the area that such fires will cover.

Whether or not fires will start depends upon the presence or absence of causative agencies during the period in which fires can start. These agencies may be classified as follows:

Human agencies: Campers (including campfires and fires caused by smokers and hunters), lumbering operations, railroads, brush burning, incendiaries, and miscellaneous.

Natural agencies: Lightning.

The area that will be burned over depends upon a large number of factors and subfactors, which may be outlined as follows:

1. Inflammability determines rate of spread, and depends upon the character of:

a. Cover, including timber, undergrowth, and litter, all of which furnish the fuel for fires. Inflammability of timber depends upon the species, age, density, and uniformity of the stand; and the condition of the stand, including such points as the presence of catfaces, moss on the trunks and lower branches, and standing dead snags. Inflammability of the undergrowth depends upon its character (grass, weeds, brush, or tree reproduction), amount (density and height), and uniformity of distribution. Inflammability of the litter is determined by its character (duff, dead grass and herbage, needles, twigs, cones, branches, logging slash, windfalls), its amount, and its condition as to dryness, decay, compactness, etc.

b. Climate and weather, which not only have much to do with determining the character and condition of the cover, but also influence directly the action of fires. The important climatic factors are: (1) Precipitation, both annual and seasonal, especially its amount and distribution during the dry seasons; (2) temperatures, means and maxima, especially during dry parts of the year; (3) humidity, including fogs, dews, etc., during the dry seasons; (4) evaporation, affecting the rate of drying of inflammable material; (5) soil moisture; (6) wind direction and velocity during the dry season.

c. Topography, which with climate practically determines the character of cover, and also directly affects the spread of fires, by the degree of slope, by the aspect, by the uniformity of terrain, and by the absolute and relative altitude, which influence atmospheric factors.

2. Controllability determines whether fires can be extinguished while small, or whether they will burn over large areas. It depends upon:

a. Men and equipment available to fight fires.

b. Accessibility—the time required to detect and to reach a fire, together with the routes and possible methods of travel.

c. Topography and soil, which influence the speed and cost of control work, such as trenching. Natural breaks, such as cliffs, streams, and bare ridges, and artificial breaks, such as roads and fire lines, should be considered here, as may also the availability of water for use on the fire line.

d. Type of forest and ground cover, which influence the method of attack, as well as the speed and cost of the work.

e. Degree of efficiency with which suppression work is carried on.

Even though a given area of forest may burn over, it does not necessarily follow that all or even a major part of the values on the burned area will be destroyed. The chance of destruction, which may be called the loss ratio, or the destructibility, depends upon the susceptibility of the various resources to direct and indirect fire damage, and also upon the intensity of the fire. These in turn depend upon

several factors, chief of which is the type of cover. The type of cover involves kind, age, density, and condition of the timber and other cover, and the amount and condition of duff and litter. The kind and depth of soil is also important, because of its relation to forest productivity as well as to the watershed protection values. All of the factors listed above under inflammability influence the intensity of the fire, and determine whether or not it will be a surface, ground, or crown fire.

COST OF SUPPRESSION

Cost of suppression may also be considered as a resultant of fire hazard, since it is determined very largely by the same factors that govern the inflammability and controllability. It is also affected by variation in wage rates and in costs of tools and subsistence, and by differences in degree of efficiency.

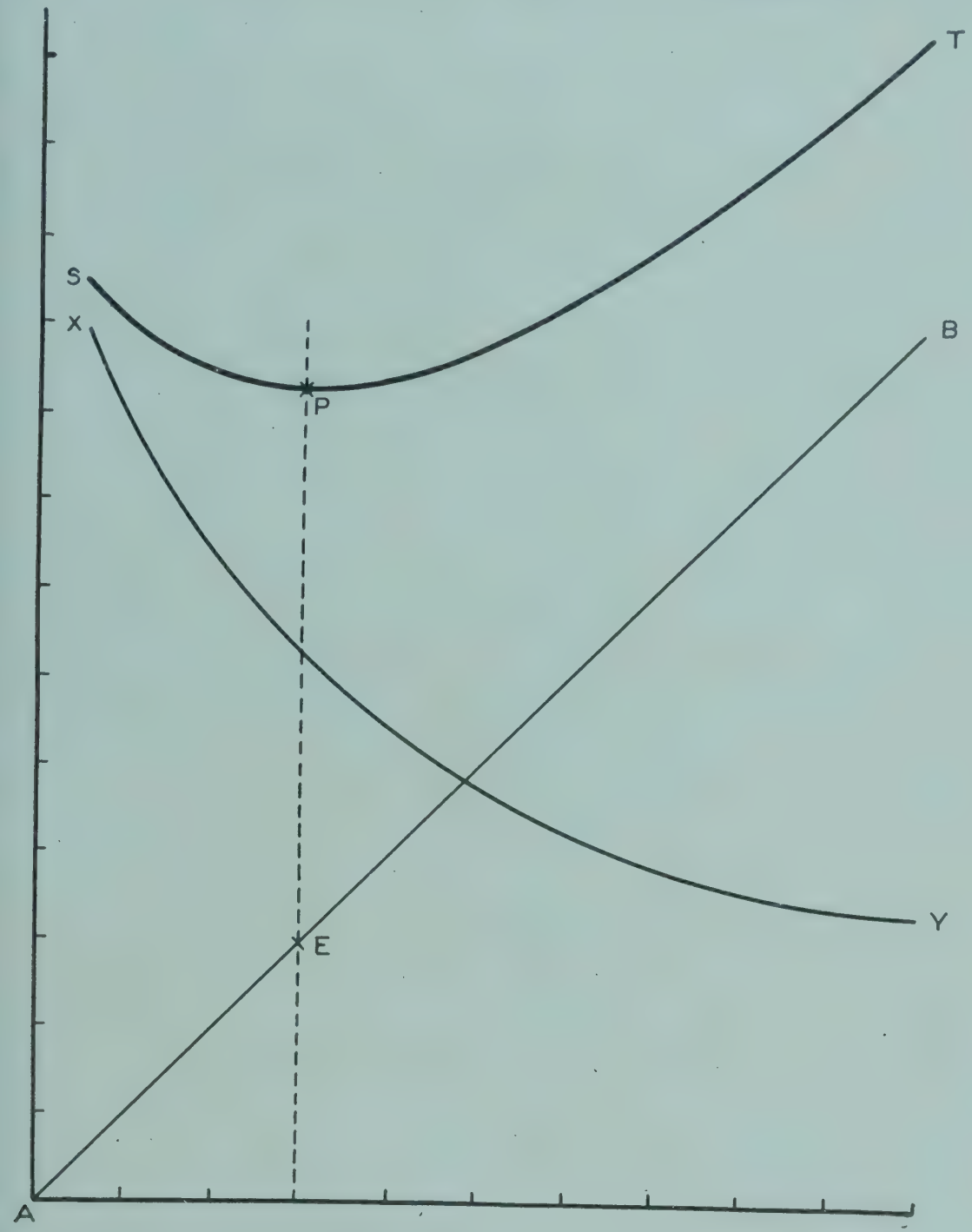


FIG. 1.—AB represents primary protection cost
XY represents sum of suppression cost plus losses "or total liability"
ST represents sum of AB plus XY
E marks the point of proper primary protection cost, where sum of all costs plus losses is at its lowest point P

COST OF PRIMARY FIRE PROTECTION

Against the sum of loss plus suppression cost or total liability, we must balance protection cost—what for the sake of convenience has been termed primary protection, to distinguish it from the cost of fire suppression. This is the known quantity—the amount that is figured in advance when a definite organization is developed to prevent, detect, and control fires. There is a certain overlapping here, unless we leave out of consideration in the suppression cost the services rendered by the primary protection organization already provided for in advance. It was not practicable to make this separation in the present study; hence the suppression costs as determined, and given in the accompanying tables, include some of the cost of the primary protection organization. The more intensive the organization, the greater will be the proportion of fires handled by it without calling on outside help; consequently the real saving in liability with decrease in hour-control will tend to be somewhat greater than the differences between suppression costs indicate. The cost of primary protection will be determined by the length of the period during which it is in effect, which depends upon the length of danger season, by the number of men, and amount of equipment used for the purpose, and by salary rates and costs of maintaining equipment.

BASIS FOR STUDY

The problem of developing a method for rating hazard and liability requires study of the relations between the various combinations of factors that may be found in different units, and of the results in losses plus suppression costs. The only scientific basis for such a study is what has actually happened, that is, the actual fire history of the different forest areas. For this purpose, the present study made use of the available records of individual fires that occurred on the national forests during the period 1911–1915 (summarized in Table I). Records previous to 1911 are too incomplete or inaccurate to be useful, and those for years after 1915 were not available at the time the study was undertaken. Records for some national forests for some of the years between 1911 and 1915 are missing. The records for subsequent years should be studied in addition to those already used, to follow up the methods for rating hazard and liability outlined in the following pages. Figures based on 10 or more years should be much more reliable than those based on only 5 years, not only because the longer period gives a much better average than does the shorter (and it is known that climatic conditions were more dangerous and fires more numerous and destructive during the 5 years following 1915), but also because the later records are more complete and accurate than the earlier ones. Only fires that burned on national forest land were used, because the records of others are less complete and, for several reasons, not comparable. Moreover, without data regarding the areas of different forest types on private lands both within and outside the forest, it would be impossible to relate either numbers of fires or areas burned over to the total acreage exposed to fire danger. (See Tables II and III.)

The records do not give detailed information regarding most of the factors whose effect it is desired to study, and even if such data were available, it seems probable that to consider them all separately would so complicate the problem that it could not be solved. Even if methods for rating could be worked out, to apply them would require us to rate nearly every individual

TABLE I.—*Fires on national forest land (1911–1915) used as a basis for study*

Region	Fires due to general risk			Fires due to special risk		
	Number of fires	Area burned	Average area per fire	Number of fires	Area burned	Average area per fire
		Acres	*Acres*		*Acres*	*Acres*
1	56	4,474	79.9	103	215	2.1
2	382	32,858	86.0	236	350	1.5
3	904	26,008	28.8	155	309	2.0
4	326	27,992	85.9	6	95	15.8
5	82	15,431	188.2	20	680	34.0
6	1,984	77,823	39.2	17	39	2.3
7	723	33,246	46.0	20	79	4.0
8	714	83,306	116.7	16	595	37.2
9	170	9,199	54.1	27	46	1.7
10	218	18,063	82.9	3	3	1.0
11	406	20,703	51.0	103	2,234	21.7
12	108	359	3.3	80	418	5.2
13	120	1,748	14.6	101	1,385	13.7
14	53	2,832	53.4	1	15	15.0
15	117	15,415	131.8			
16	1,093	56,515	51.7	100	3,418	34.2
17	605	148,141	244.9	38	2,582	68.0
18	1,308	121,913	93.2	154	1,435	9.3
19	250	4,637	18.5	113	2,200	19.5
20	187	22,224	118.8			
21	121	25,674	212.2	181	5,638	31.1
Totals	9,927	748,561	75.4	1,474	21,736	14.7

TABLE II.—*Approximate areas of forest types by regions* [a]

Region	Areas in thousands of acres by forest types													
	Western yellow pine	Western yellow pine, sugar pine, incense cedar	Douglas fir and larch	Lodgepole (and knobcone) pine	Spruce	Fir, hemlock, and cedar	White pine	Subalpine	Aspen	Pinon-juniper and oak-digger pine	Hardwood	Brush	Grass and sage	Total net national forest area
1	48	------	477	1,108	66	------	------	412	------	------	----	(b)	383	2,745
2	891	------	1,837	1,957	315	------	133	1,230	------	------	----	(b)	325	6,948
3	272	------	1,882	217	188	69	1,128	689	------	------	----	(b)	553	5,079
4	906	------	1,229	409	240	301	------	807	------	------	----	(b)	128	4,390
5	2	------	3,571	123	------	1,655	------	1,887	------	------	----	1,331	(b)	8,470
6	597	1,303	1,935	124	------	346	------	95	------	210	----	1,615	97	6,441
7	(b)	2,352	183	620	------	402	------	340	------	477	----	413	427	5,296
8	3,539	------	2,843	2,207	366	77	------	1,511	1	------	----	498	1,200	12,561
9	2	------	952	2,214	86	------	------	917	26	------	----	(b)	1,408	6,076
10	35	------	1,156	3,510	576	------	------	1,399	550	18	----	(b)	3,757	12,470
11	1,448	------	------	------	6	------	------	------	------	------	----	(b)	348	1,803
12	859	------	65	440	836	------	------	16	40	45	----	20	156	2,850
13	2	------	28	1,512	1,297	------	------	(b)	1,051	35	----	212	797	5,298
14	206	------	327	615	646	------	------	117	806	831	----	838	654	5,352
15	183	------	43	102	60	339	------	(b)	490	2,925	----	866	2,425	7,816
16	865	1,720	60	333	------	455	------	447	------	213	----	1,143	129	6,308
17	------	336	111	------	------	------	------	6	------	------	144	2,726	39	3,376
18	5,433	------	(b)	------	(b)	------	------	(b)	(b)	3,259	----	5,179	(b)	13,871
19	4,739	------	(b)	230	(b)	------	------	(b)	743	713	----	1,598	(b)	8,682
20	578	------	(b)	------	(b)	------	------	(b)	------	2,409	----	554	(b)	3,541
21	------	------	------	543	130	80	160	------	(b)	------	185	(b)	30	1,128
Total	20,605	5,711	16,699	[c]16,264	4,812	3,724	[d]1,421	9,873	3,707	11,135	329	16,993	12,856	130,501

[a] These are in many cases based on very rough estimates.
[b] Indicates area included with some other type. Totals for each region do not in most cases agree with sums of figures for separate types, because barren areas are also included in totals (sometimes barren is included in brush, grass, or subalpine).
[c] Includes 543,000 acres jack pine in Region 21. [d] Includes 160,000 acres white and red pine in Region 21.

TABLE III.—*Percentages of total areas of different types burned over each year; averages for 5-year period, 1911–1915* [a]

Region	Commercial timber types								Nonmerchantable types [b]						All types
	Western yellow pine	Western yellow pine, sugar pine, incense cedar	Douglas fir and Douglas fir-larch	Lodgepole pine	Spruce, cedar, hemlock, and firs	White pine	Hardwoods	Total commercial timber types	Subalpine	Aspen	Pinon-juniper and digger pine-oak	Chaparral and brush	Grass and sage	Total	
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1	(c)	------	0.007	0.066	------	------	------	0.045	(c)	------	------	------	0.034	0.015	0.033
2	0.067	------	.199	.099	0.047	0.053	------	.125	0.009	------	------	------	.017	.010	.095
3	.037	------	.076	.007	.475	.198	------	.133	.025	------	------	------	.004	.016	.102
4	.128	------	.441	.108	.418	------	------	.345	.012	------	------	------	1.418	.044	.245
5	------	------	.139	------	.515	------	------	.210	.016	------	------	0.318	------	.281	.246
6	.063	0.273	.497	.163	.305	------	------	.304	(e)	------	0.081	.494	.137	.390	.329
7	------	.278	------	.255	.047	------	------	.242	.012	------	.001	.337	.278	.141	.196
8	.440	------	.210	.108	(c)	------	------	.236	.015	------	------	.003	.354	.114	.207
9	------	------	.041	.031	.168	------	------	.038	.002	------	------	------	.066	.042	.037
10	.146	------	.043	.009	.009	------	------	.018	(c)	------	------	------	.066	.050	.031
11	.257	------	------	------	------	------	------	.257	------	------	------	.116	------	.116	.230
12	.006	------	.008	(c)	(c)	------	------	.003	(e)	0.005	------	------	.002	.002	.003
13	------	------	.079	.008	.003	------	------	.006	------	.004	------	------	.016	.009	.007
14	.003	------	(c)	(c)	(c)	------	------	(c)	------	(e)	(c)	.042	------	.020	.012
15	.015	------	.003	.001	(c)	------	------	.004	------	(e)	.009	.017	.112	.046	.040
16	.265	.079	------	(e)	.021	------	------	.110	.021	------	.656	.452	.663	.395	.196
17	.269	------	.870	------	------	------	0.022	.322	.003	------	------	.985	2.122	.999	.878
18	.385	------	------	------	------	------	------	.385	------	------	.024	.018	------	.020	.180
19	.014	------	------	.002	------	------	------	.014	------	------	.001	.006	------	.005	.011
20	.438	------	------	------	------	------	------	.438	------	------	.050	.128	------	.064	.125
21	------	------	------	[d].683	(c)	.574	.145	.445	------	------	------	----	.817	.817	.455

United States average: 86,878 acres out of 55,971,000 acres timber=0.155 per cent; 58,718 acres out of 47,839,000 acres noncommercial=0.123 per cent; 145,596 acres out of 103,810,000 for all types=0.140 per cent.

[a] Based on incomplete records for some regions, especially Washington and Oregon.
[b] Open and woodland types often combined; figures given apply to two or more types combined.
[c] Less than $\frac{1}{1000}$ of 1 per cent. [d] Jack pine. [e] None burned.

TABLE IV.—*Important climatic characteristics of the several national forest subregions* [a]

Region	Precipitation			Temperature			Length of growing season	Prevailing winds during fire season	Characteristic types of forest
	Total annual (average and range)	July and August	Rainy days (over 0.01 inch)	Mean annual	Mean maximum	Days per year above 90° F.			
	In.	*In.*	*No.*	*° F.*	*° F.*	*No.*	*Days*		
1	13(10–15)	2.0	95	44	55	6–13	150	W., SW	LP.
2	16(15–20)	2.0	100	43	54	1–2	130	W., SW	YP, WL–DF.
3	33(25–40)	2.5	120	43	55	5–6	115	S., SW	WP.
4	14(10–30)	0.7	60	47	58	8–16	120	S	YP, WL–DF.
5	64(37–140)	1.7	150	51	60	1–11	210	W., SW	DF.
6	44(35–70)	1.8	115	53	65	0–8	200	NW	DF, redwood.
7	14(8–23)	0.6	65	46	62	8–39	130	S., NW. [b]	YP.
8	16(15–35)	0.8	70	49	61	4–34	135	S., NW. [c]	YP.
9	13(10–15)	1.2	65	41	55	1–17	[d] 90	W., NW	LP, DF.
10	15(10–20)	1.7	75	39	52	0–6	[e] 85	S., SE.; W., SW. [f]	LP.
11	17(12–25)	5.0	75	44	55	5–29	150	N., NW.; E., SE. [g]	YP.
12	13(10–15)	2.0	60	46	62	2–29	125	N.; SE. [h]	YP, LP.
13	15(9–27)	2.7	90	39	55	0–20	100	N	LP, ES.
14	12(8–16)	1.5	60	48	62	12–47	140	SW., W	DF, ES.
15	9(7–12)	1.5	50	46	62	19–51	[i] 115	SW., S	Pinon, juniper.
16	49(35–80)	2.2	70	50	(*j*)	(*j*)	120	S., W., NW	YP–SP–IC.
17	14(10–20)	1.6	35	62	70	1–15	300	W., NW	Chaparral.
18	14(8–18)	[k] 1.4	60	54	70	16–51	160	E., S, SW	YP.
19	12(7–16)	[k] 2.1	55	50	66	2–79	[l] 155	W., SW	YP.
20	13(9–16)	[k] 1.1	55	62	75	46–80	230	N., NW	Woodland, YP.
21	32(27–35)	[m] 4.0	[n] 90–120	[o] 37–41	[p] 49–52	1–10	[q] 105–130	N., S., SW.; SE. [r]	WP, jack pine.

[a] Data based on Bulletin Q, Weather Bureau, United States Department of Agriculture.
[b] S. in southern part, NW. at north end.
[c] NW. and S. in western part, S. and SW. in east.
[d] Range, 55 to 120 days.
[e] Range, 65 to 105 days.
[f] SE. and S. in northwest, W. and SW. in south.
[g] E. and SE. in May.
[h] SE. in May, July, August, September.
[i] Range, 95 to 130 days.
[j] Data lacking.
[k] Figures are for April–May–June.
[l] Range, 130 to 180 days.
[m] Figures are for October–November.
[n] Minnesota, 90 days; Michigan, 120 days.
[o] Minnesota, 37°; Michigan, 41°.
[p] Minnesota, 49°; Michigan, 52°.
[q] Minnesota, 105 days; Michigan, 130 days.
[r] Minnesota, N. in spring, S. and SW. summer and fall; Michigan, SE. in spring and fall.

acre separately. If, however, numbers of factors can be grouped together in such a way as to reduce the number of items that must be considered in rating, and so as to make it possible to use the data concerning the history of past fires, which are already at hand or obtainable, it may be possible to develop a method that can be applied. This involves the principle of classification of risks, somewhat analogous to that used in the insurance business, where rating is based on the probable losses for a class of risks, rather than for each individual risk separately.

CLASSIFICATION OF RISKS

The object of such a classification should be to throw together into one class all forest tracts whose factors of risk are so substantially similar that the probable fire loss and suppression cost per unit of area, over a period of years, will be fairly uniform. Because of the nature of the data available for study, and also in order that ratings can be applied in working out actual protection organizations, the classification must be along rather broad lines, with a minimum of detail. Accordingly, the following general scheme was followed:

1. To allow for general difference in climatic and seasonal factors and the resultant differences in general forest conditions, the western national forest region, exclusive of Alaska, was divided into 21 subregions, mainly on the basis of climatic characteristics. (See Table IV and figs. 2 and 3.) These subregions are as follows:

1. Northern Rocky Mountains.
2. Western Montana.
3. Northern Idaho.
4. Eastern Washington.
5. Western Washington and Oregon.[2]
6. Southern Oregon and Northern California Coast Ranges.
7. Southeastern Oregon and northeastern California.
8. Eastern Oregon and southwestern Idaho.

[2] The study did not cover Alaska or the eastern national forests.

9. Central Idaho and southwestern Montana.
10. Yellowstone plateau region.
11. Black Hills and eastern Montana.
12. Eastern Colorado.
13. Northwestern Colorado and southern Wyoming.
14. Wasatch and Uinta Ranges.
15. Interior desert region—mostly Nevada.
16. West slope of Sierras.
17. Southern California.
18. Colorado Plateau region.
19. Northern New Mexico and southwestern Colorado.
20. Southern Arizona and New Mexico.
21. Lake States.[2]

values, fire hazard, and cost of suppression. The grouping of types within each region is shown in Table V. Such a classification is crude, it is realized, since it does not allow for such factors as age of stand or for the wide local variations in inflammability of individual stands of a given type, due to such factors as the presence of logging slash or other débris. Ratings obtained, therefore, will represent aver-

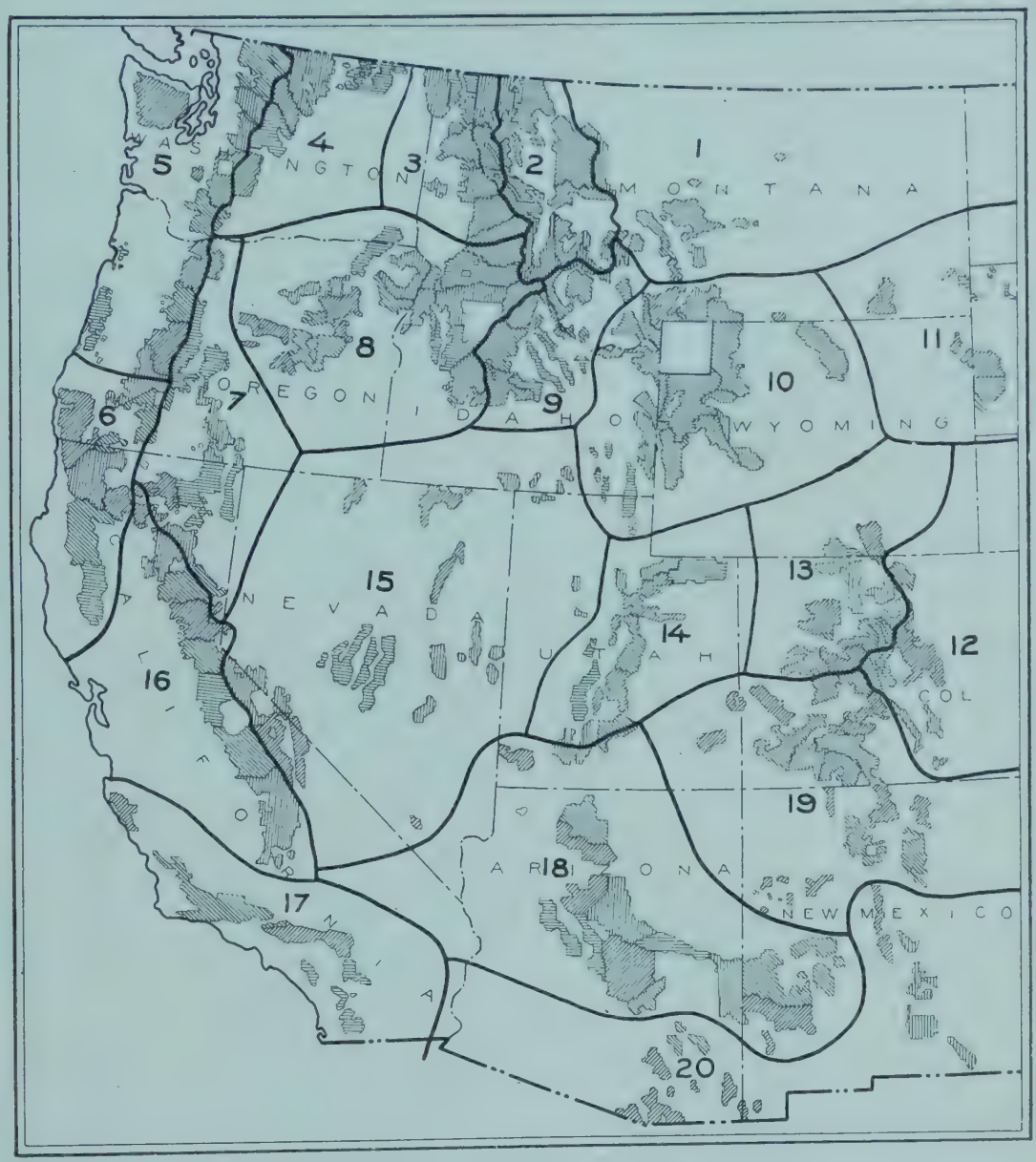
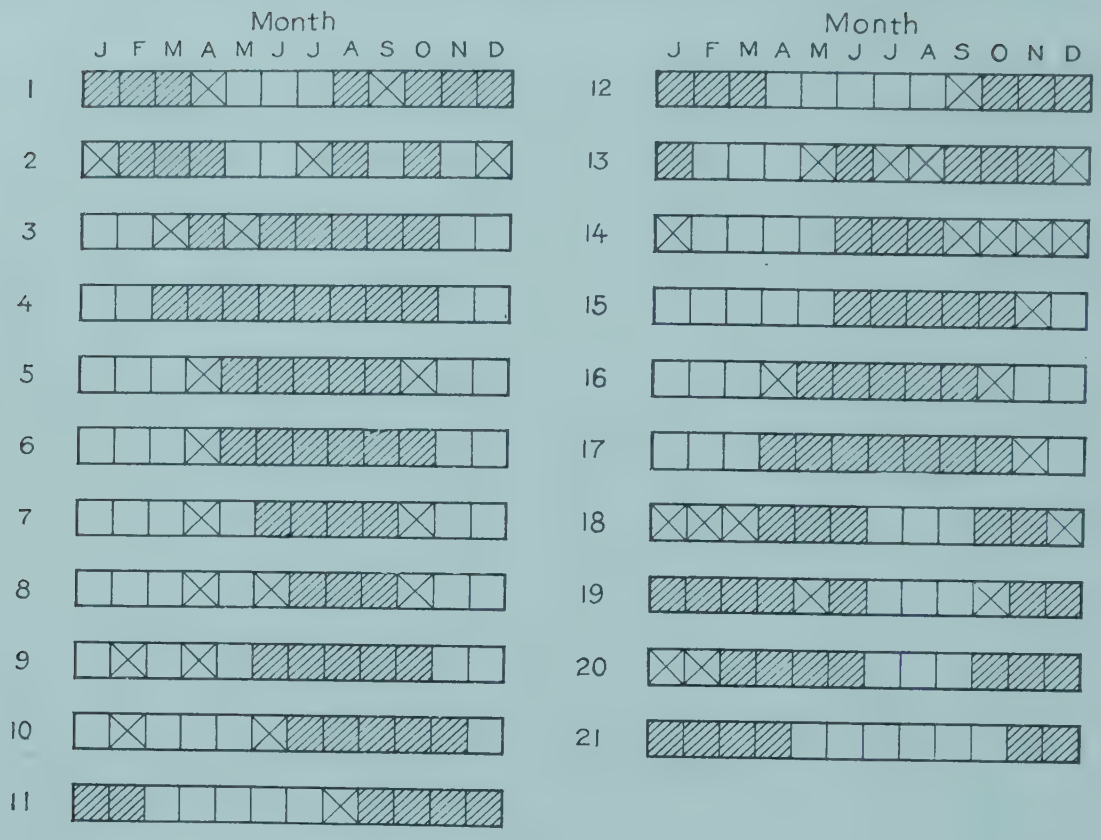


FIG. 2.—Subregions used in studying fire hazard. (Region 21, Lake States, not shown)

2. Within each of these regions it seems reasonable to assume that a given type of forest is in a broad way fairly uniform in its composition and general characteristics, so that within the region classification of the forest areas according to type of cover will in a general way allow for variations in the factors which determine total and destructible ages of fairly broad application, but may not show what can be expected on individual small units. These factors can be allowed for only when the fire records and the inventory of our forest resources include information concerning them. It is hoped that this can be done in future work.

[2] The study did not cover Alaska or the eastern national forests.



LEGEND

▨ – Months in which less than one-twelfth of the annual precipitation falls

⊠ – Months in which about one-twelfth of the annual precipitation falls

☐ – Months in which more than one-twelfth of the annual precipitation falls

Numbers refer to the regions outlined in Table V.

FIG. 3.—Distribution of precipitation by months, for the several forest regions

TABLE V.—*List of subregions and forests, with the classification of forest types* [a]

Region	Forests	Forest types	Representative weather stations
1. Northern Rocky Mountains.	Lewis and Clark, Helena (except west end), Jefferson, Gallatin (northern part), Absaroka (northern part).	Douglas fir, lodgepole pine, subalpine (including Engelmann spruce), open (grass, sage, etc.).	Helena, Great Falls.
2. Western Montana.	Blackfeet, Bitterroot, Flathead, Missoula, Helena (W.), Deerlodge (N.), Kootenai (E. of range 31 W.), Cabinet (E. of range 29 W.), Lolo (E. of line from NE. of T. 19–28 to NW. of T. 17–29).	Western yellow pine, Douglas fir–western larch, lodgepole pine, spruce–cedar–hemlock–white fir, western white pine, subalpine, open (grass, sage, etc.).	Kalispell, Missoula.
3. Northern Idaho.	Kaniksu, Pend Oreille, St. Joe, Coeur d'Alene, Kootenai (W.), Clearwater, Cabinet (W.), Selway (Lochsa drainage), Lolo (W.).	Same as Region 2, with "open" separated into "brush" and "grass."	Priest River, Porthill, Murray, Wallace, Sandpoint, Spokane.
4. Eastern Washington.	Colville, Okanogan, Chelan, Wenatchee, Rainier (E.), Columbia (E.).	Same as Region 3, except that western white pine not used.	Ellensburg, Colfax, Lakeside, Spokane, Lyle.

[a] Common and botanical names for the species listed here and mentioned elsewhere in this article are as follows: Western yellow pine (*Pinus ponderosa*), sugar pine (*P. lambertiana*), jack pine (*P. banksiana*), western white pine (*P. monticola*), lodgepole pine (*P. contorta*), eastern white pine (*P. strobus*), red or Norway pine (*P. resinosa*), Jeffrey pine (*P. jeffreyi*), pinon pine (*P. edulis*), digger pine (*P. sabiniana*), Douglas fir (*Pseudotsuga taxifolia*), bigcone spruce (*Ps. macrocarpa*), lowland white fir (*Abies grandis*), red fir (*A. magnifica*), balsam (*A. balsamea*), Engelmann spruce (*Picea engelmanni*), white or eastern spruce (*P. canadensis*), black spruce (*P. mariana*), western larch (*Larix occidentalis*), tamarack (*L. laricina*), western hemlock, (*Tsuga heterophylla*), juniper (*Juniperus* spp.), redwood (*Sequcia sempervirens*), incense ceder (*Libocedrus decurrens*), red cedar (*Thuja plicata*), aspen (*Populus tremuloides*).

TABLE V.—*List of subregions and forests, with the classification of forest types*—Con.

Region	Forests	Forest types	Representative weather stations
5. Western Washington and Oregon.	Mt. Baker, Snoqualmie, Olympic, Rainier (W.), Columbia (W.), Mt. Hood, (W.), Umpqua (N.), Santiam, Siuslaw, Cascade.	Lower slope (Douglas fir, cedar, hemlock, spruce, etc.), upper slope (true firs, hemlock, etc.), subalpine.	Olympia, Snohomish, Centralia, Portland, Seattle, Albany, Glenora.
6. Southwest Oregon and northwest California.	Umpqua (S.), Crater (W.), Siskiyou, Klamath, Trinity, California, Shasta (W.).	Western yellow and sugar pine, Douglas fir, red and white firs, subalpine, oak and digger pine, brush fields, grass.	Ashland, Roseburg, Eureka, Sisson, Ukiah, Weaverville.
7. Southeastern Oregon and northeastern California.	Oregon (E.), Deschutes, Fremont, Crater (E.), Modoc, Shasta (E.), Lassen (E.), Plumas (E.), Tahoe, (E.).	Western yellow and sugar pine, lodgepole pine, fir, subalpine, woodland, brush, grass and sage.	Prineville, Dayville, Lakeview, Silver Lake, Cedarville, Susanville, Reno.
8. Eastern Oregon and southwestern Idaho.	Ochoco, Malheur, Umatilla, Wallowa, Whitman, Minam, Wenaha, Nez Perce, Selway (S.), Weiser, Idaho, Payette, Boise, Salmon (N. and W.), Sawtooth (W.).	Same as Region 2, except no white pine.	Walla Walla, Pomeroy, Baker, Joseph, Payette, Boise.
9. Central Idaho and southwestern Montana.	Sawtooth (E.), Salmon (E.), Challis, Lemhi, Beaverhead, Deerlodge (S.), Madison (N.).	Douglas fir, lodgepole pine, Engelmann spruce, subalpine, brush, grass and sage.	Butte, Soldier.
10. Yellowstone Plateau.	Madison (S.), Gallatin (S.), Beartooth, Absaroka (S.), Shoshone, Bighorn, Bonneville, Bridger, Washakie, Teton, Targhee, Palisade, Wyoming, Caribou, Cache, Pocatello.	Same types as Region 9	Henrys Lake, Yellowstone Park, Thayne, Lander.
11. Black Hills and eastern Montana.	Black Hills, Harney, Sioux, Custer.	Western yellow pine, open (brush and grass).	Miles City, Oakdale, Spearfish.
12. Eastern Colorado.	Colorado, Pike, San Isabel, Leadville (S.).	Western yellow pine, Douglas fir, lodgepole pine, Engelmann spruce, subalpine, open (brush, grass, and woodland).	Fort Collins, Denver. Colorado Springs, Salida, Saguache, San Luis.
13. Northwestern Colorado and southern Wyoming.	Hayden, Routt, White River, Sopris, Battlement, Medicine Bow, Arapahoe, Holy Cross, Leadville (N.)	Douglas fir, lodgepole pine, Engelmann spruce, subalpine, aspen, brush, grass.	Laramie, Cheyenne, Rawlins, Walden, Meeker, Pagoda, Breckenridge.
14. Wasatch and Uinta Ranges.	Ashley, Uinta, Manti, Powell (N.), Fillmore, Fishlake, Sevier (N.), Wasatch (exc. W.).	Western yellow pine, Douglas fir, lodgepole pine, Engelmann spruce, subalpine, aspen, pinon-juniper, brush, grass, and sage.	Vernal, Provo, Salt Lake.
15. Interior desert region (Nevada).	Minidoka, Humboldt, Santa Rosa, Nevada, Wasatch (W.), Mono, Ruby, Toiyabe, Moapa, Inyo.	Western yellow pine, lodgepole pine, Douglas fir, red and white fir, pinon-juniper, brush and chaparral (including aspen and mahogany), grass and sage.	Winnemucca, Potts, Elko, Ely, Oakley, Idaho, Independence, Calif.
16. West slope of Sierras.	Sequoia, Stanislaus, Tahoe (W.), Lassen (W.), Sierra, Eldorado, Plumas (W.), Shasta (S., center.)	Western yellow and sugar pine, red and white fir, lodgepole and knobcone pines, subalpine, oak and digger pine, brush fields, grass and open.	Auburn, Sisson, Summit, Laporte, Yosemite, Quincy.
17. Southern California.	Monterey, Santa Barbara, Angeles, San Bernardino, Cleveland.	Western yellow and Jeffrey pine, fir and pine slopes, subalpine, hardwood bottoms, chaparral, grass, sage, desert.	San Diego, Redlands, Los Angeles, Santa Barbara, Santa Cruz, San Bernardino.
18. Colorado Plateau.	Sevier (S.), Powell (S.), Coconino, Prescott, Apache, Datil, Crook (N.), Dixie (except Moapa), Kaibab, Tusayan, Tonto, Sitgreaves, Gila.	Western yellow pine, Douglas fir mixed, spruce and subalpine, pinon-juniper, brush and aspen, sage and grass.	Holbrook, Prescott, Fort Apache, Fort Bayard.
19. Southwestern Colorado and northern New Mexico.	LaSalle, Gunnison, Rio Grande, Durango, Carson, Manzano (W.), Uncompahgre, Cochetopa, Montezuma, San Juan, Santa Fé.	Same as Region 18, with addition of lodgepole pine at north end.	Moab, Utah; Durango, Colo.; Aztec, N. Mex.; Santa Fé; Fort Wingate.
20. Southern Arizona-New Mexico.	Manzano (E.), Coronado, Lincoln-Alamo, Crook (S.).	Same as Region 18	Mesilla Park, Tucson, Dudleyville, Fort Huachuca.
21. Lake States	Minnesota, Superior, Michigan.	Eastern white and red pines, jack pine, spruce, balsam, tamarack, hardwoods, grass and open.	Sault Ste. Marie, Park Rapids, Grayling, Mount Iron.

3. The causes of fire may be classified as "general" or "blanket" risks, and "special" or "local," sometimes called "fixed" risks. The latter include such causes as railroads, lumbering operations, and brush burning, whose locations are definitely fixed within certain restricted known localities. Some camper fires might also be included in this class, because they are localized along established travel routes or at established camp sites. Since, however the data contained in the fire records do not permit segregation of such fires from the other camper fires, they are all thrown together with fires caused by lightning, incendiaries, miscellaneous, and unknown causes, into the general risk class, which includes those fires that may occur practically anywhere within a forest unit.

For the purpose of rating the general hazard of given regions and forest types, only these general risks were considered. Rating of special risks will have to be done for each unit individually, according to the kind, extent, and location of of the fixed causes of fire within or adjacent to it, and according to the character of forest covering the particular parts of the unit exposed to such risks.

CHARACTER OF RATING

The rating of risks for different types within each of the 21 subregions was based on the following considerations:

1. No data are available to indicate what losses might amount to without any protection whatever. It has sometimes been stated that such data would afford a good measure for justifiable protection expenditure, but such is not the case. It is more important to know, and it is possible to learn, what losses may be expected with protection of different degrees of intensity.

2. Intensity of protection can be measured best by what may be termed the "hour control"—that is, the time within which fires on a given area are reached. The larger the personnel, or the better the facilities for detection, communication, and travel, the smaller will be the hour control. Reduction of hour control may be expected to result in reduced fire loss and also in reduced suppression cost, but will in general involve also increases in the cost of primary protection, which will partly offset the saving.

3. Data on primary protection costs for different types of forest and for protection of different degrees of intensity are not available nor can they be worked out on the basis of averages, but will vary according to the particular circumstances in each individual forest unit.

4. Such general rating as can be made, therefore, will attempt to show probable fire losses and the probable costs of fire suppression, per unit of area in different forest types of the several regions, with protection of various degrees of intensity. The balancing of these liabilities against costs of maintaining the corresponding degrees of protection will not be undertaken.

CALCULATION OF BASIC DATA FOR USE IN RATING

In the first place, the records of individual fires were segregated by subregions and as far as possible by types within these regions. This could not be done in all cases, because of the incompleteness of the data contained in the original records. Each group of these records was then studied along the following lines.

RELATION BETWEEN HOUR CONTROL AND SIZE OF FIRES

The areas burned per fire were correlated with the time elapsed before control work commenced. The purpose of this was to tie in the area burned with the intensity of protective organization. Area here means final area burned over by the fire up to the time it was extinguished; the time factor used is the elapsed period between discovery of the fire and the time when actual work of suppression began. Discovery time was used rather than the time when the fires started, because the latter was seldom reported. Fires which had obviously been burning for a long time before discovery were not included in the calculations, nor were fires that occurred under especially unfavorable conditions, as evidenced by abnormally slow spread. It is well known that fires usually spread very slowly, often hardly at all, during the night, and suppression crews usually can not get to a fire as fast at night as during the day. For these reasons, in order to put all the elapsed periods on approximately the same basis, the hours between 9 p. m. and 6 a. m. were rated at only half the actual elapsed time.

Fires were grouped according to elapsed periods—e. g., less than 1 hour, 1 to 2 hours, 2 to 3 hours, etc.; and the elapsed times and final acreages for the fires in each group were averaged and plotted as abscissae and ordinates,

respectively. Curves were then drawn on the basis of these points, showing the average size that fires may be expected to attain for different elapsed periods. (See figs. 4 to 18.)

RELATION BETWEEN SIZE OF FIRES AND COSTS OF SUPPRESSION

In organizing the suppression work, it is important to know the relation between the speed of attack, which is determined by the intensity of the protective organization, and the cost of putting out fires that may occur. The cost of suppression depends more directly on the size of the fire than on the speed of attack, though the latter has much to do with determining the size of fire. Accordingly, the fires were grouped by area classes—less than one acre, one to two acres, etc.—and the average areas and average suppression costs of fires within each group were then plotted on cross-section paper, as abscissae and ordinates, respectively. From the curves based on these points it is possible to determine the probable average suppression cost for fires of any given size, and from these curves and those of size based on elapsed time, the suppression cost according to speed of attack can be ascertained (Table VI and figs. 19 to 36).

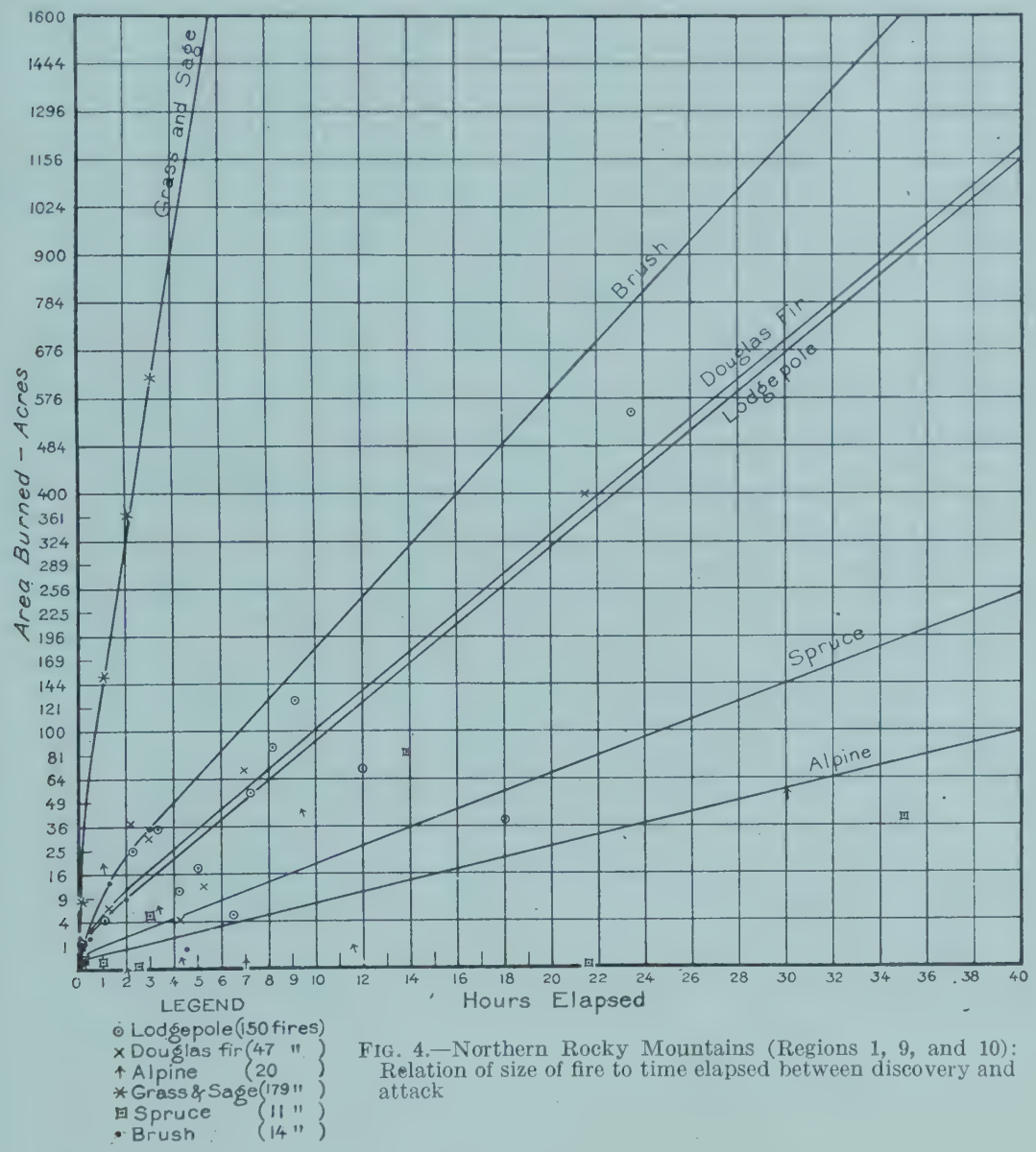


FIG. 4.—Northern Rocky Mountains (Regions 1, 9, and 10): Relation of size of fire to time elapsed between discovery and attack

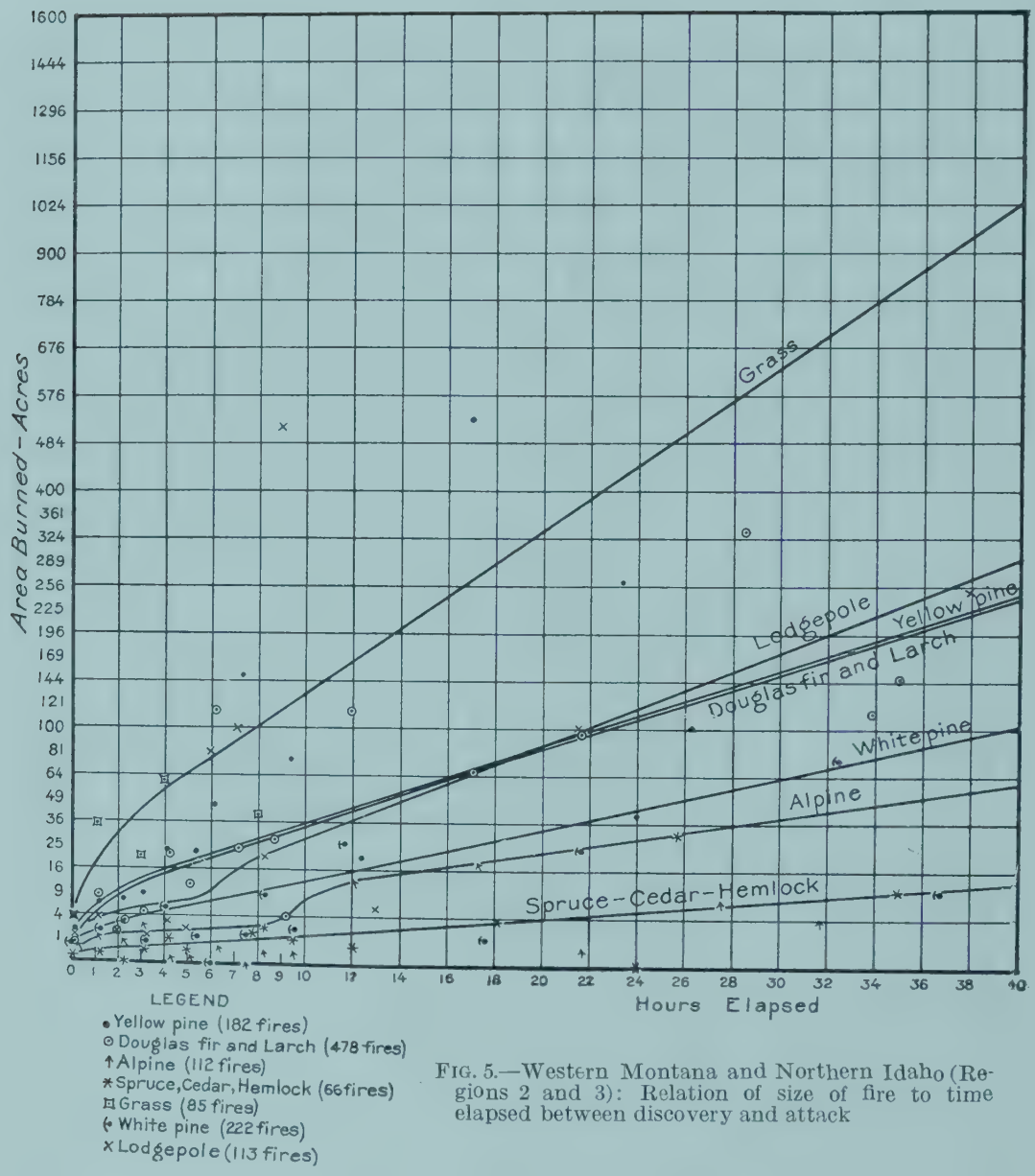


FIG. 5.—Western Montana and Northern Idaho (Regions 2 and 3): Relation of size of fire to time elapsed between discovery and attack

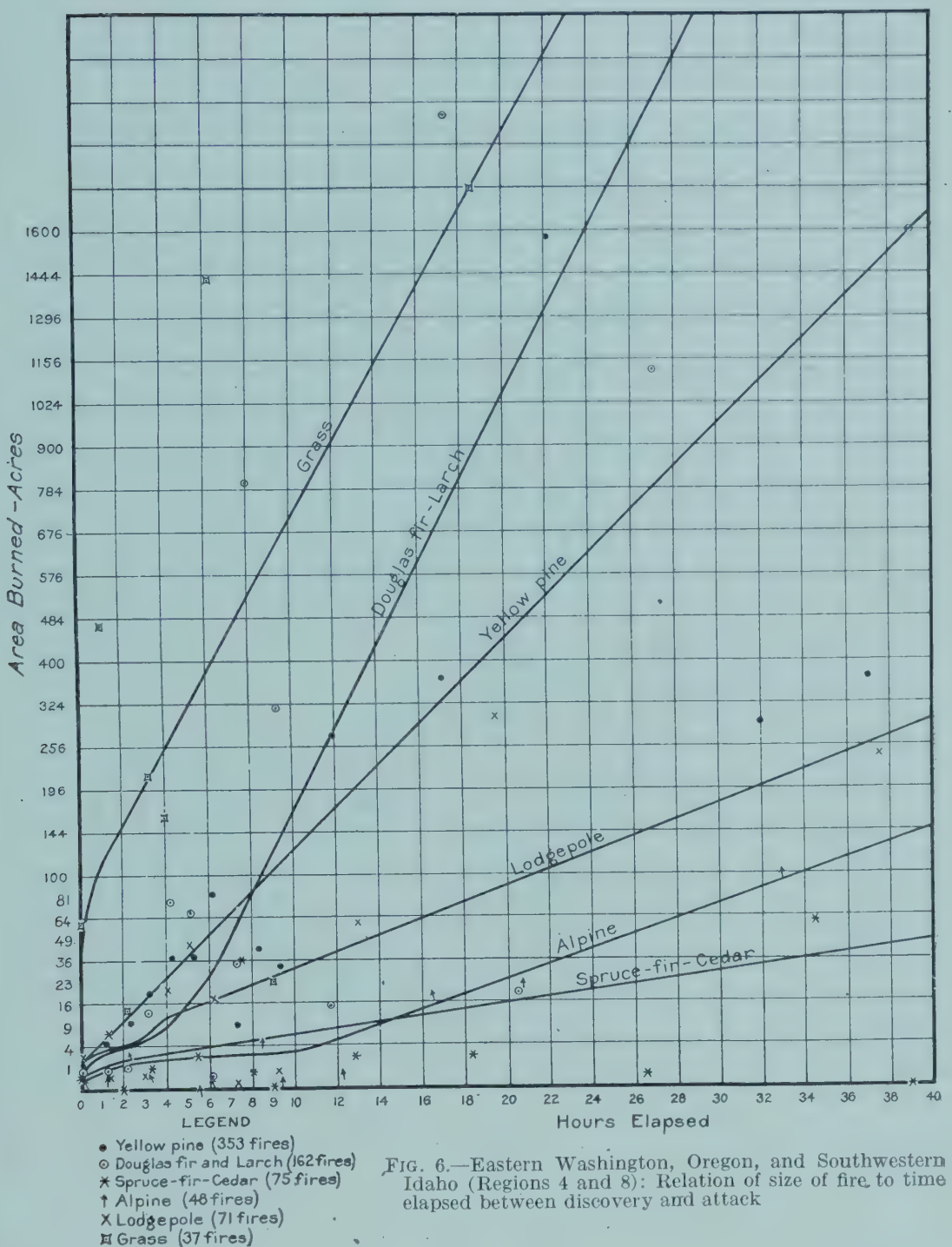


FIG. 6.—Eastern Washington, Oregon, and Southwestern Idaho (Regions 4 and 8): Relation of size of fire to time elapsed between discovery and attack

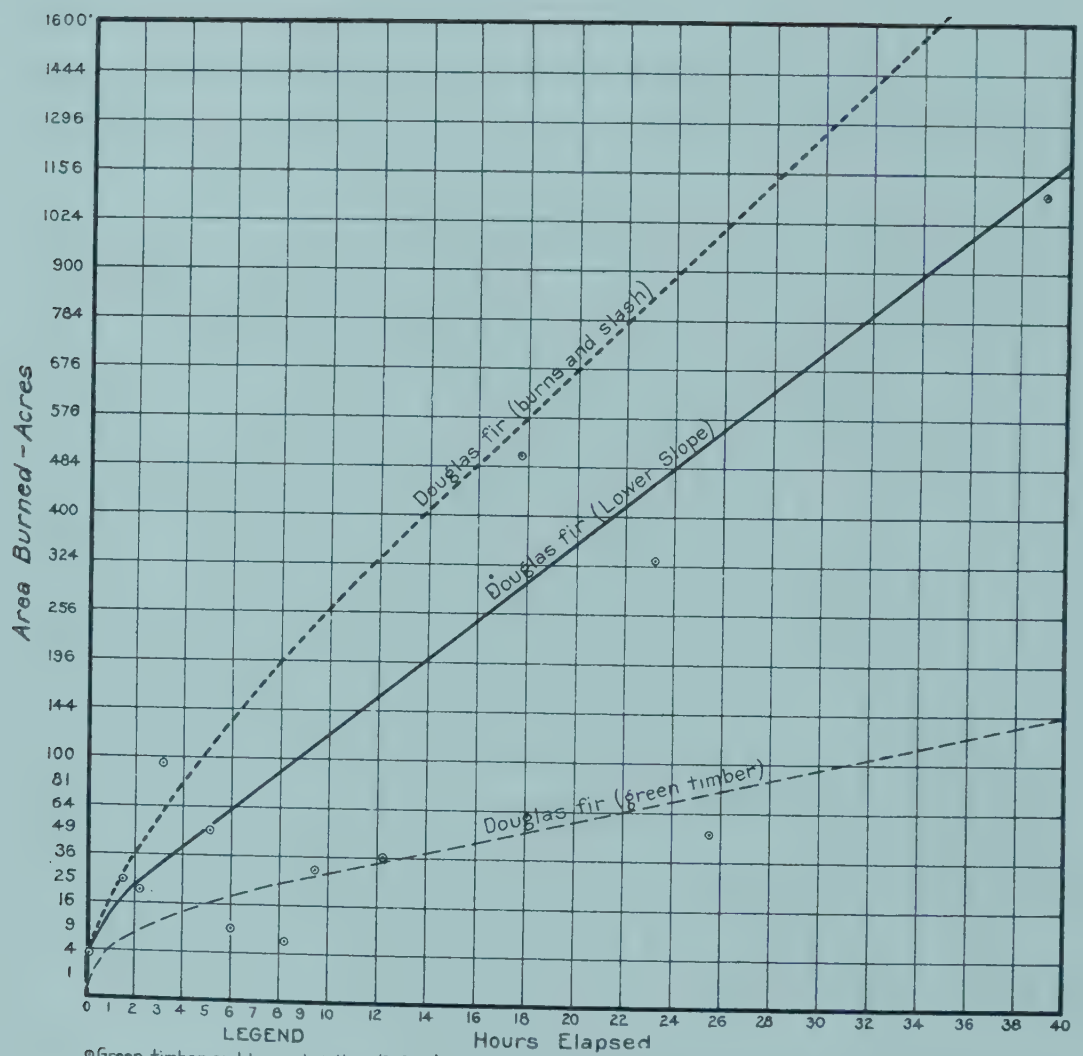


FIG. 7.—Western Washington and Oregon (Region 5): Relation of size of fire to time elapsed between discovery and attack

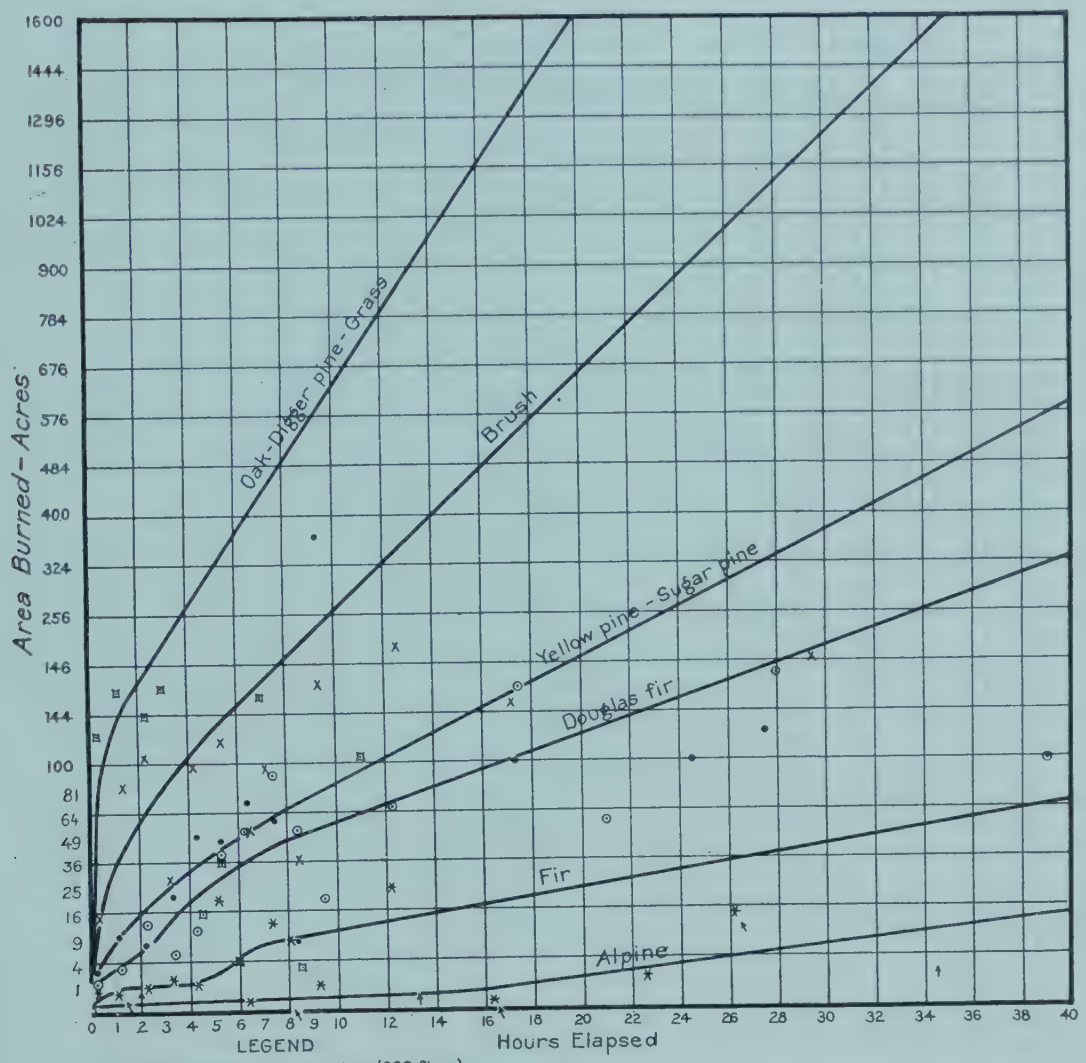


FIG. 8.—Southwestern Oregon and Northwestern California (Region 6): Relation of size of fire to time elapsed between discovery and attack

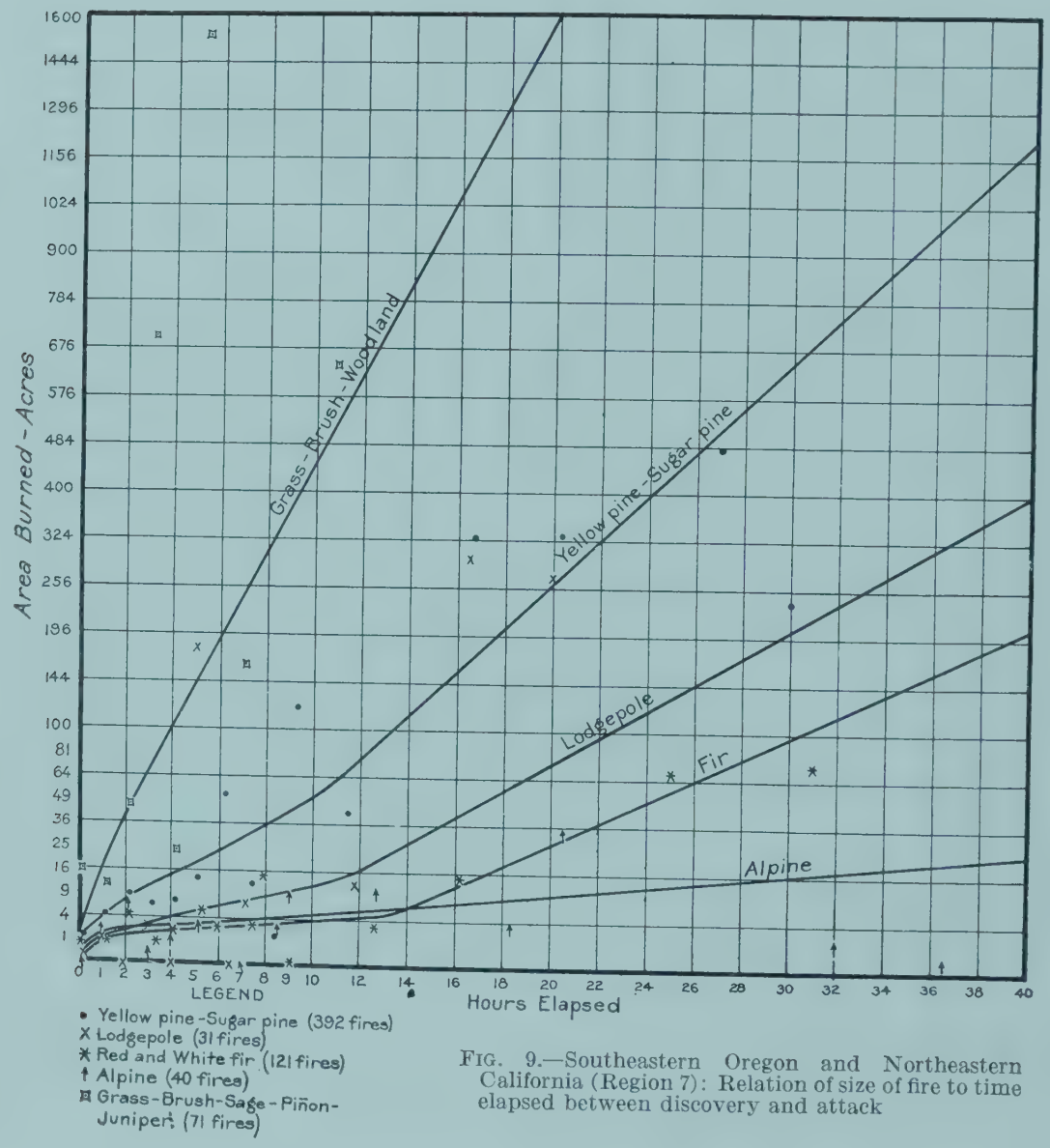


FIG. 9.—Southeastern Oregon and Northeastern California (Region 7): Relation of size of fire to time elapsed between discovery and attack

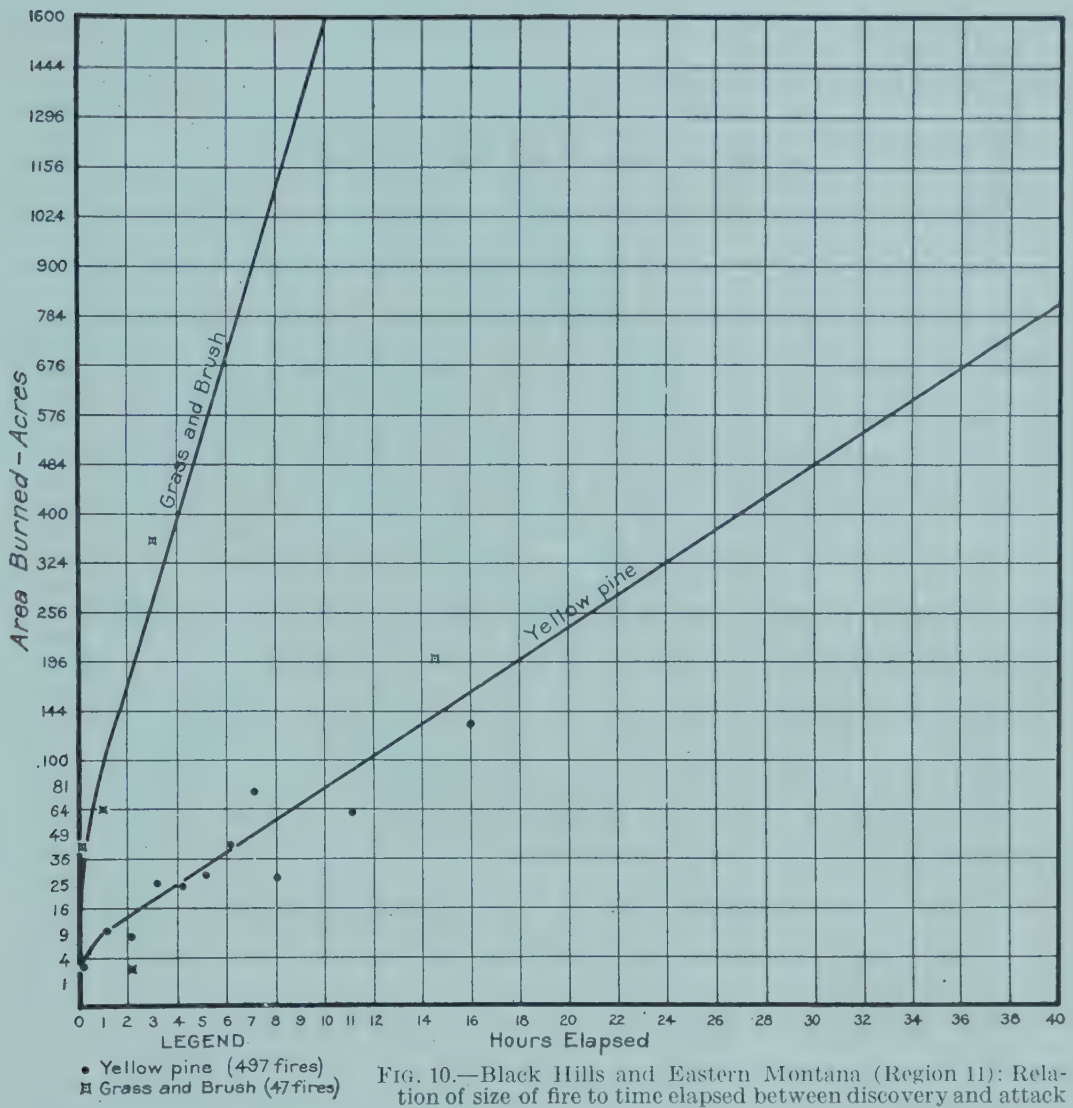


FIG. 10.—Black Hills and Eastern Montana (Region 11): Relation of size of fire to time elapsed between discovery and attack

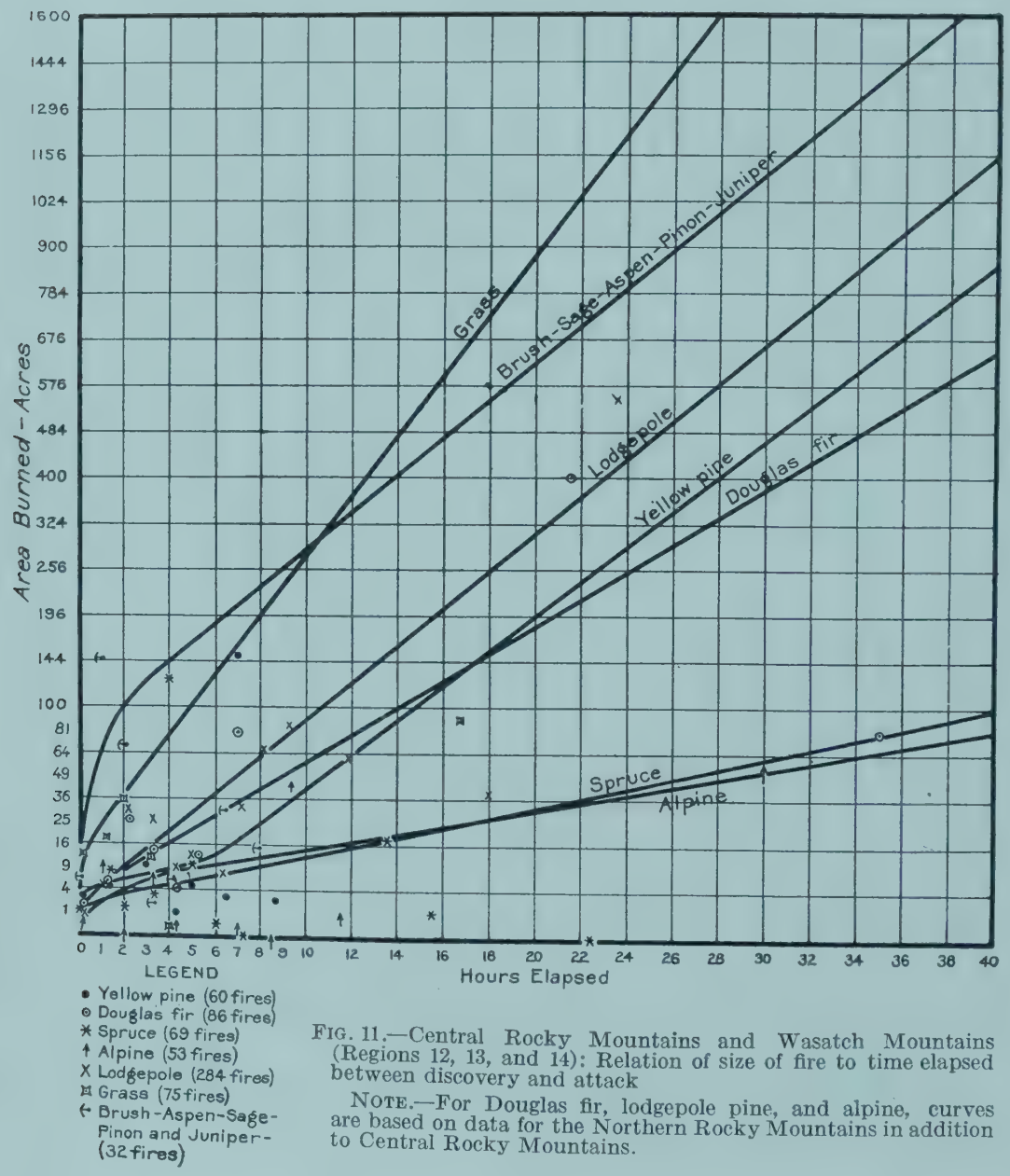


FIG. 11.—Central Rocky Mountains and Wasatch Mountains (Regions 12, 13, and 14): Relation of size of fire to time elapsed between discovery and attack

NOTE.—For Douglas fir, lodgepole pine, and alpine, curves are based on data for the Northern Rocky Mountains in addition to Central Rocky Mountains.

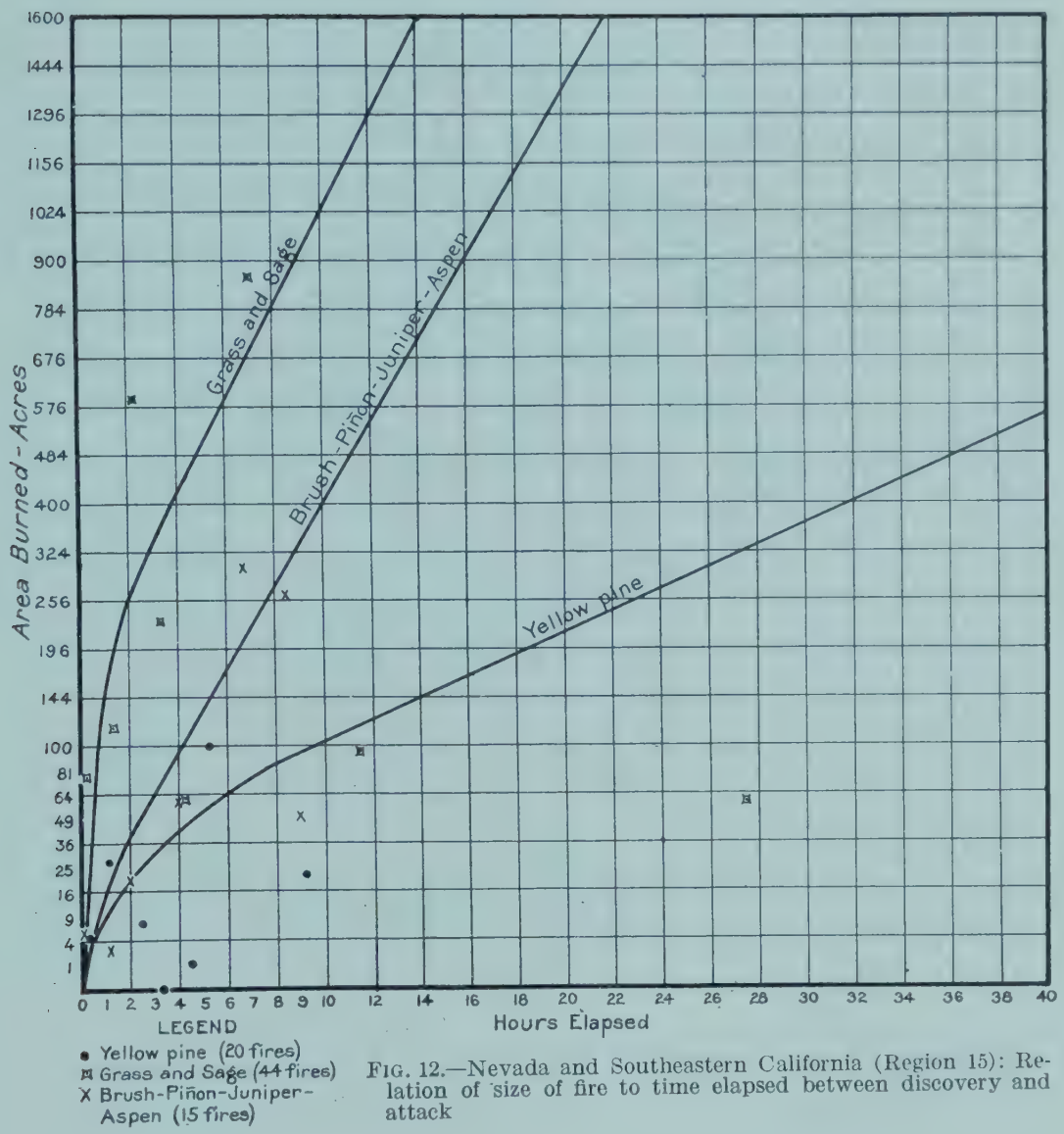


FIG. 12.—Nevada and Southeastern California (Region 15): Relation of size of fire to time elapsed between discovery and attack

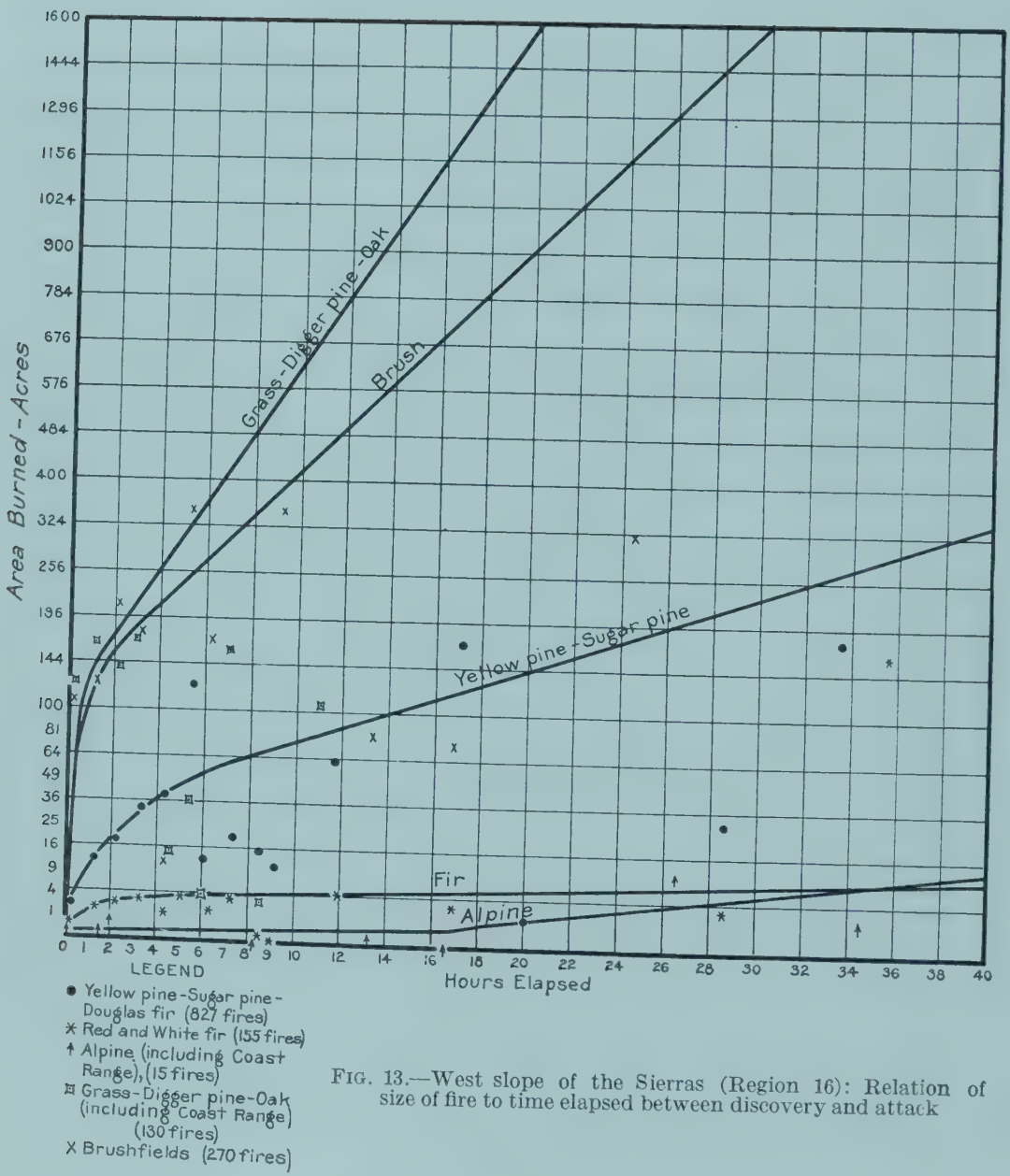


FIG. 13.—West slope of the Sierras (Region 16): Relation of size of fire to time elapsed between discovery and attack

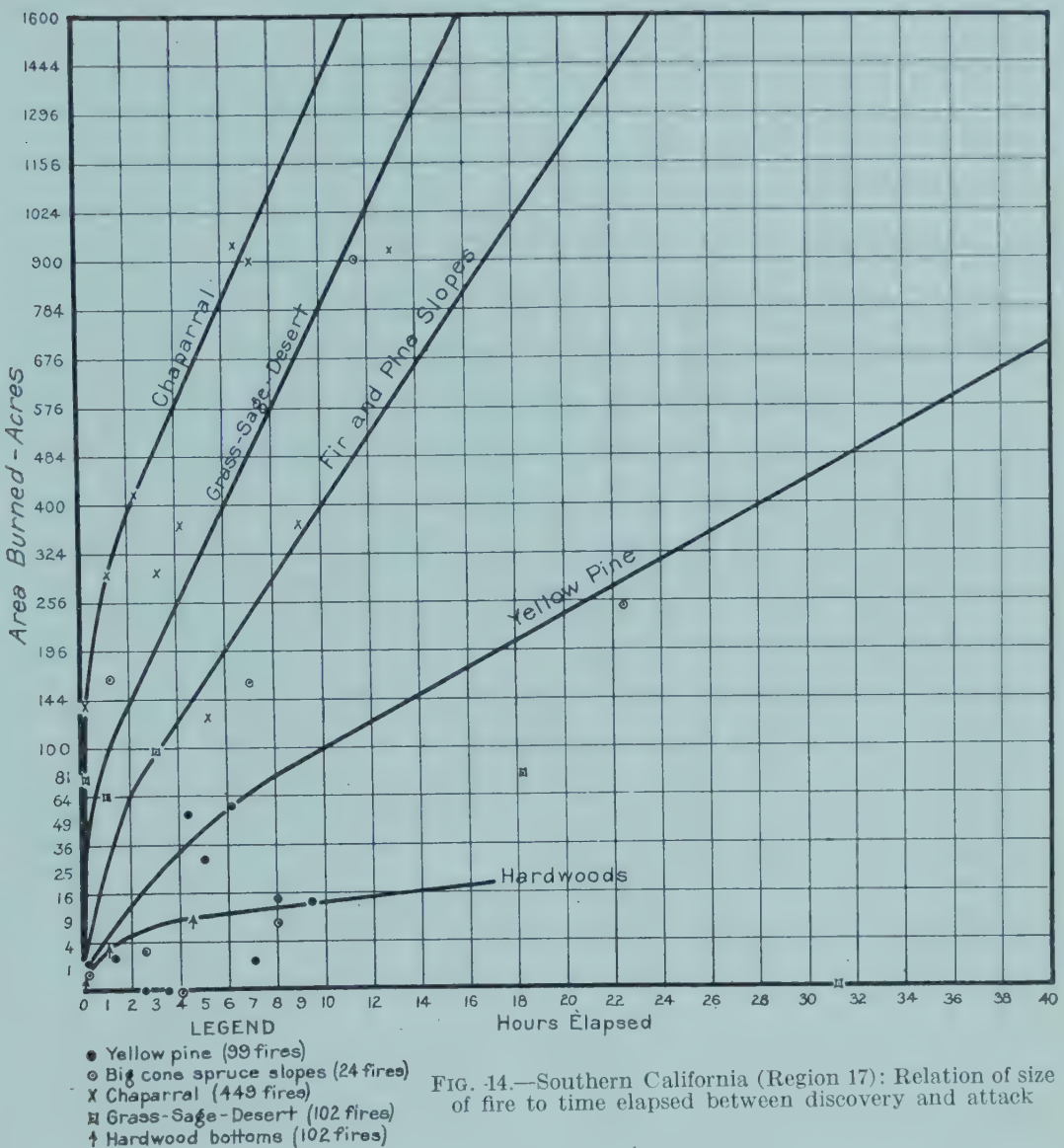


FIG. 14.—Southern California (Region 17): Relation of size of fire to time elapsed between discovery and attack

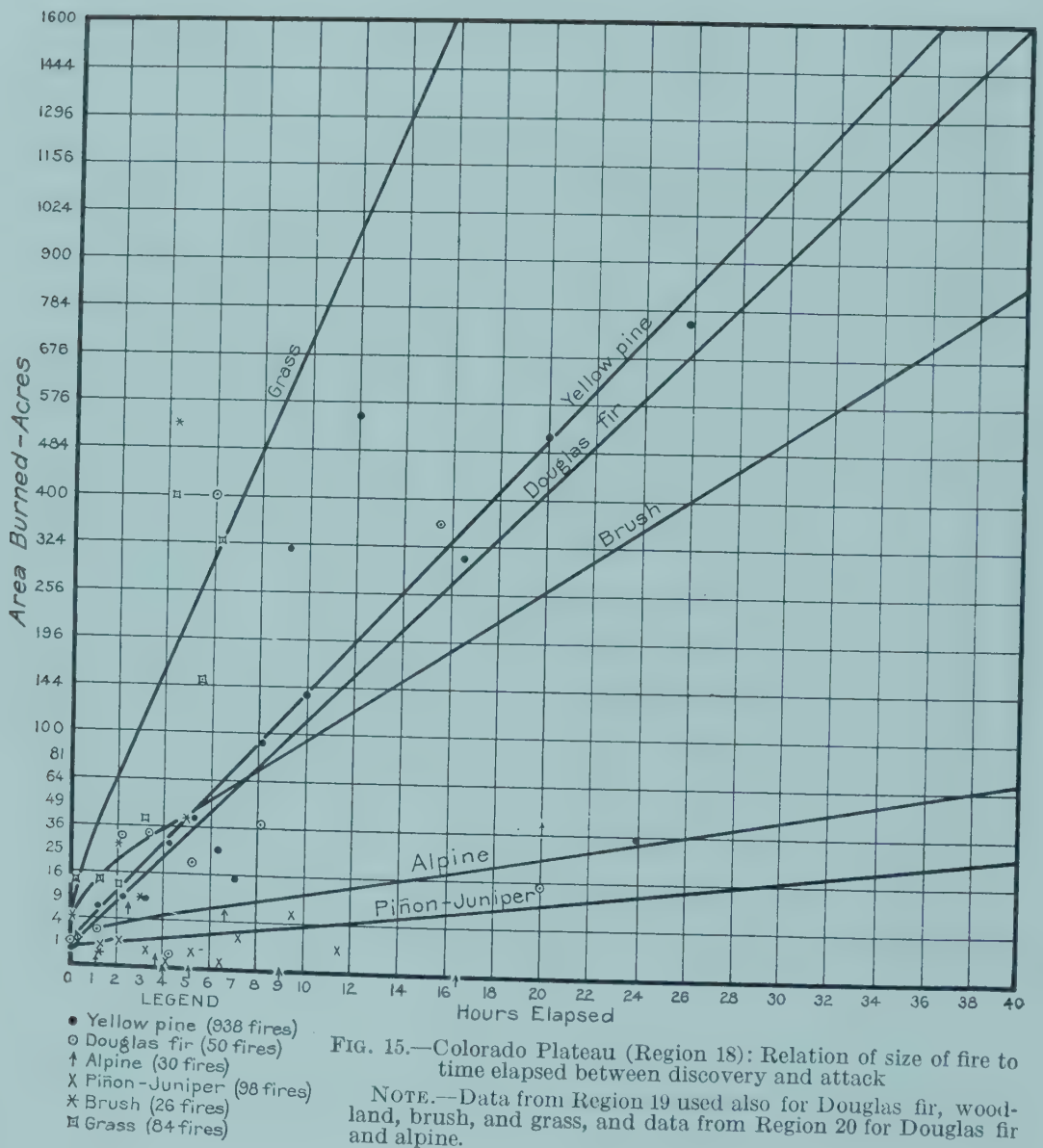


FIG. 15.—Colorado Plateau (Region 18): Relation of size of fire to time elapsed between discovery and attack

NOTE.—Data from Region 19 used also for Douglas fir, woodland, brush, and grass, and data from Region 20 for Douglas fir and alpine.

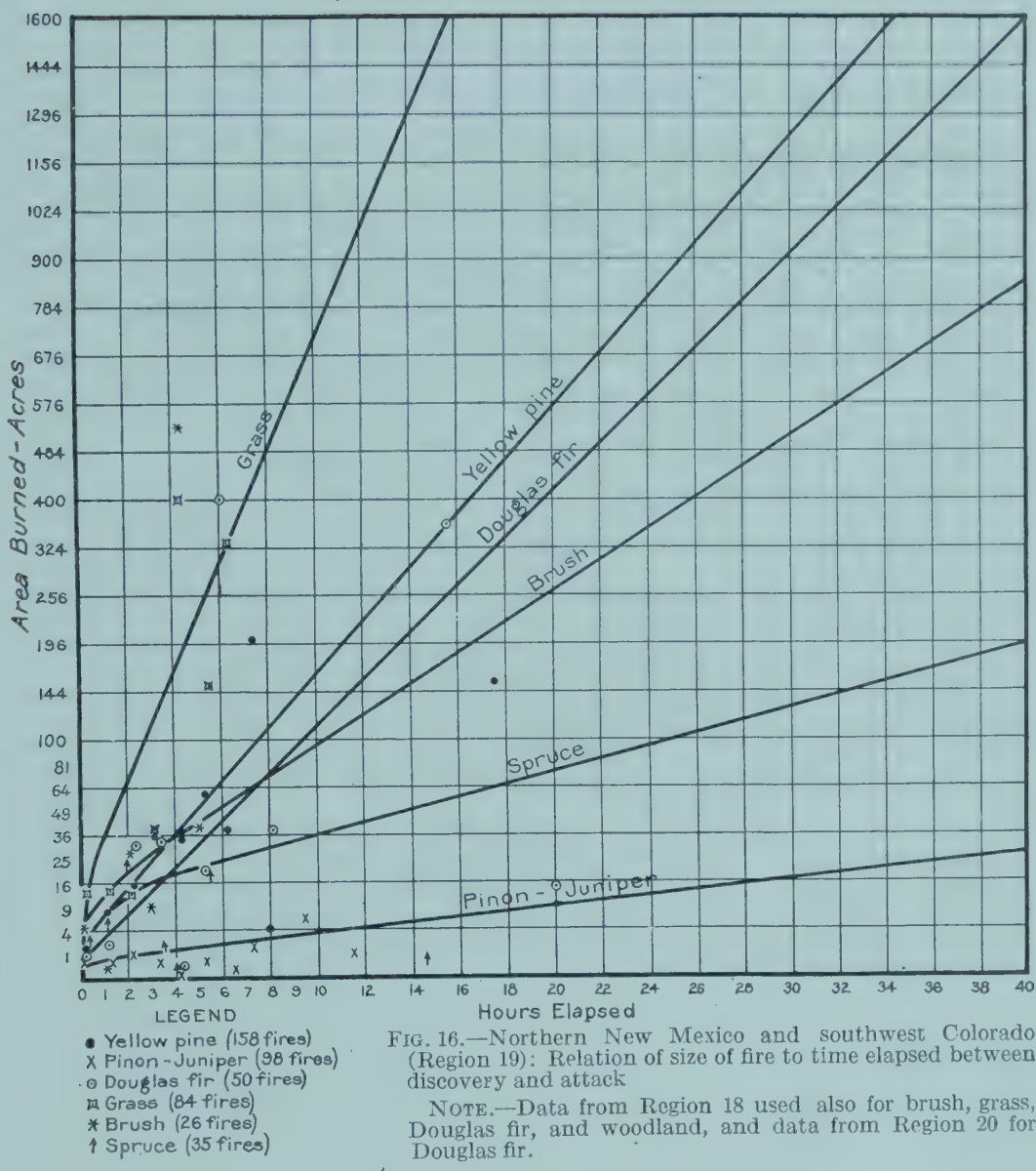


FIG. 16.—Northern New Mexico and southwest Colorado (Region 19): Relation of size of fire to time elapsed between discovery and attack

NOTE.—Data from Region 18 used also for brush, grass, Douglas fir, and woodland, and data from Region 20 for Douglas fir.

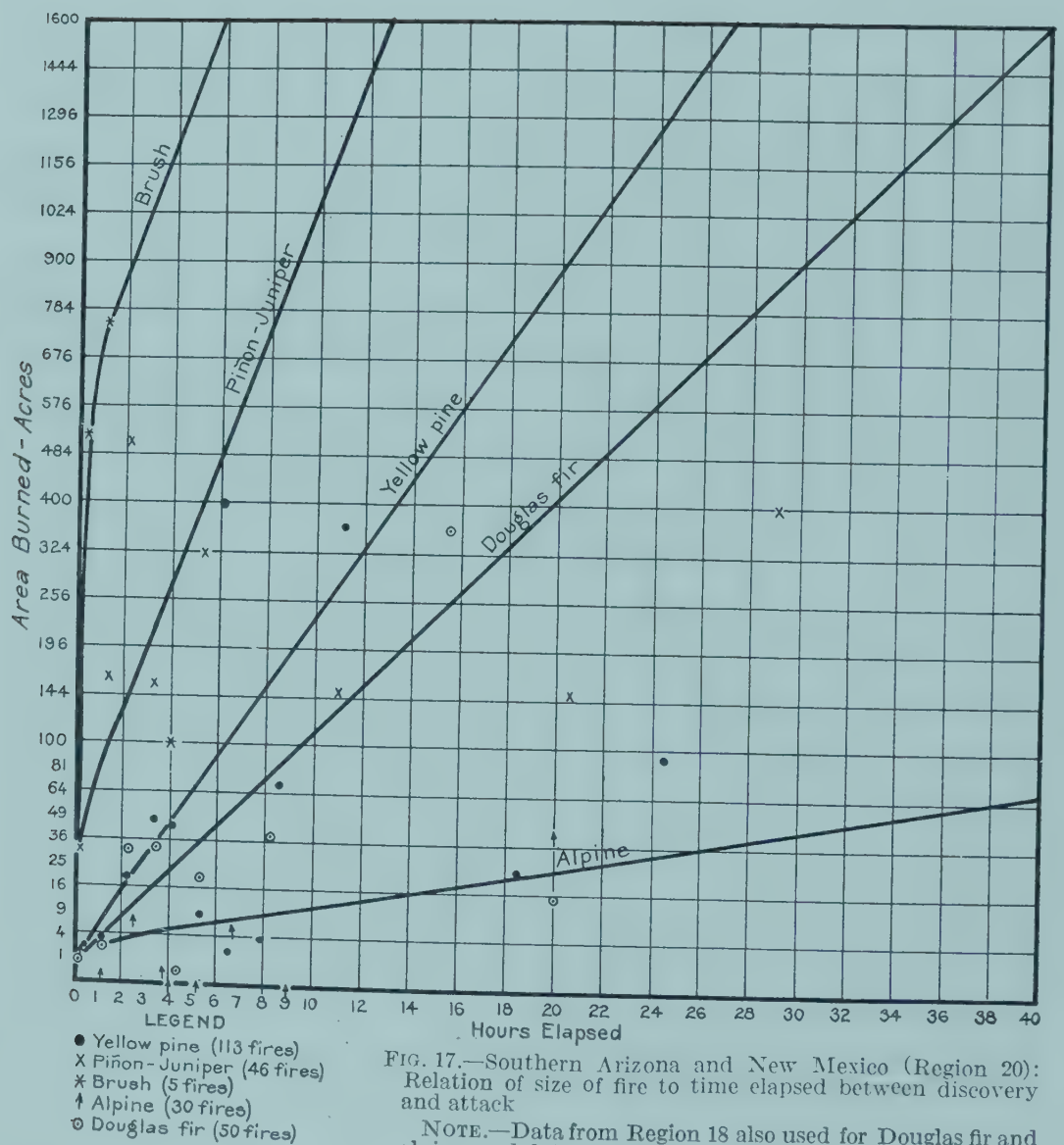


FIG. 17.—Southern Arizona and New Mexico (Region 20): Relation of size of fire to time elapsed between discovery and attack

NOTE.—Data from Region 18 also used for Douglas fir and alpine, and data from Region 19 for Douglas fir.

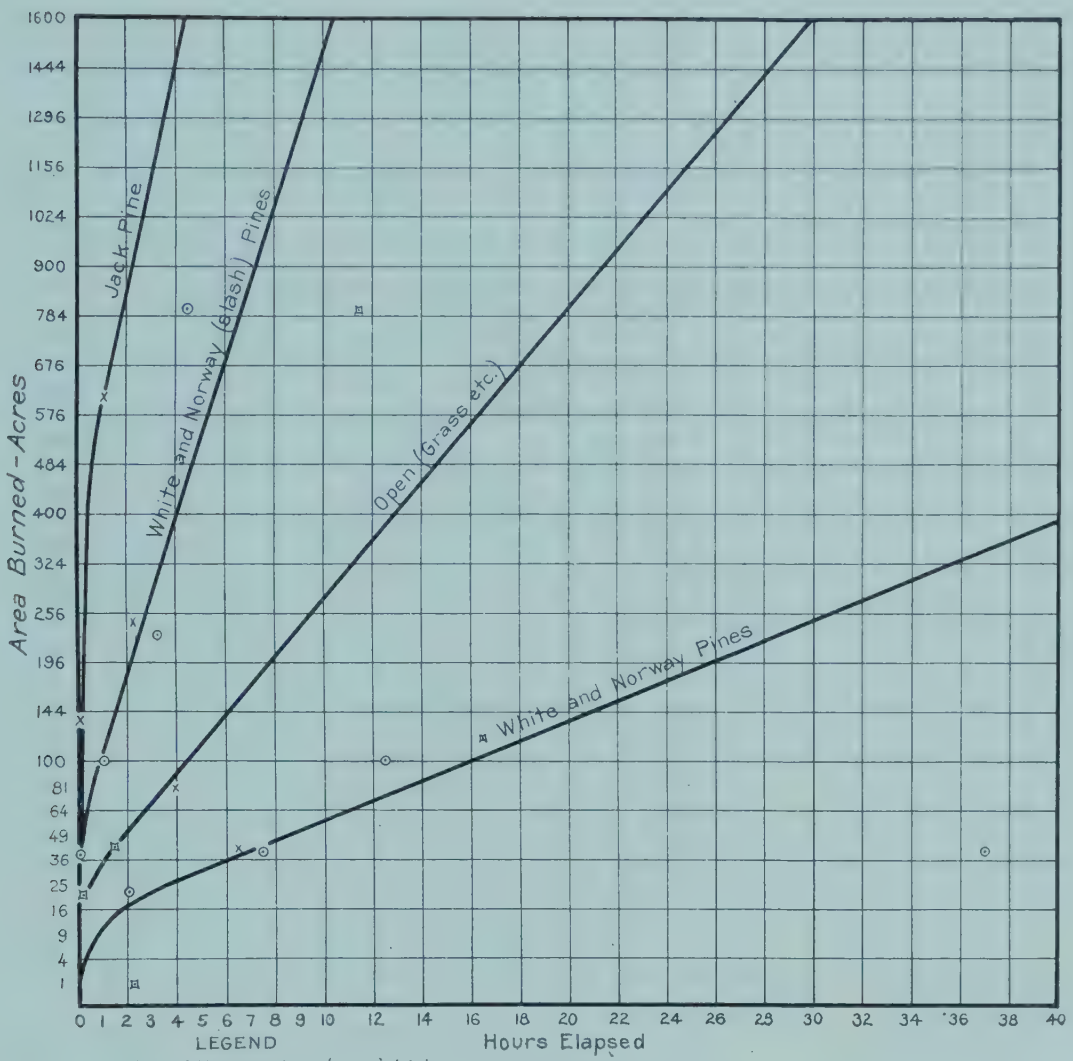


FIG. 18.—Lake States (Region 21): Relation of size of fire to time elapsed between discovery and attack

NOTE.—Not enough data for hardwoods and tamarack.

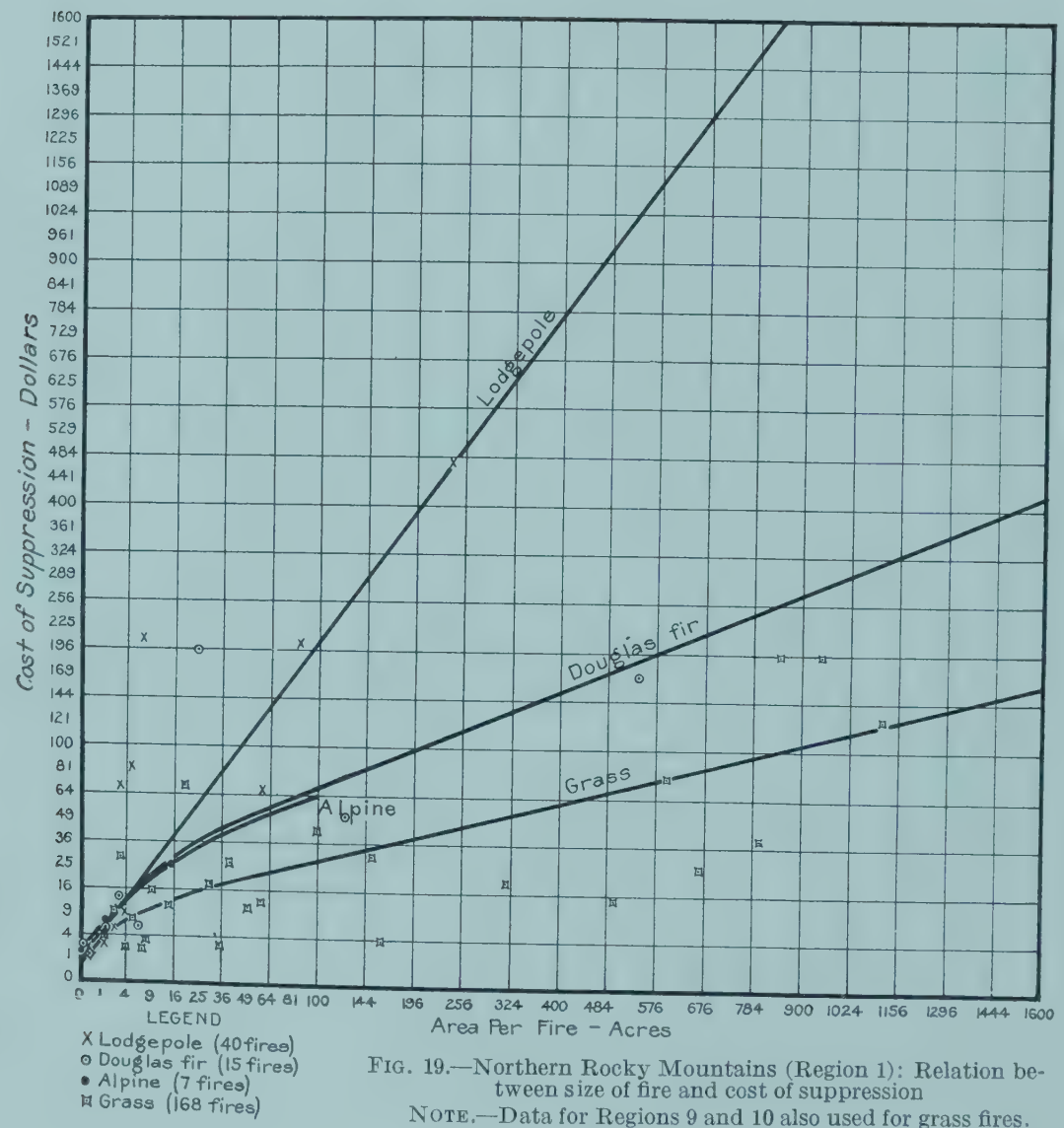


FIG. 19.—Northern Rocky Mountains (Region 1): Relation between size of fire and cost of suppression

NOTE.—Data for Regions 9 and 10 also used for grass fires.

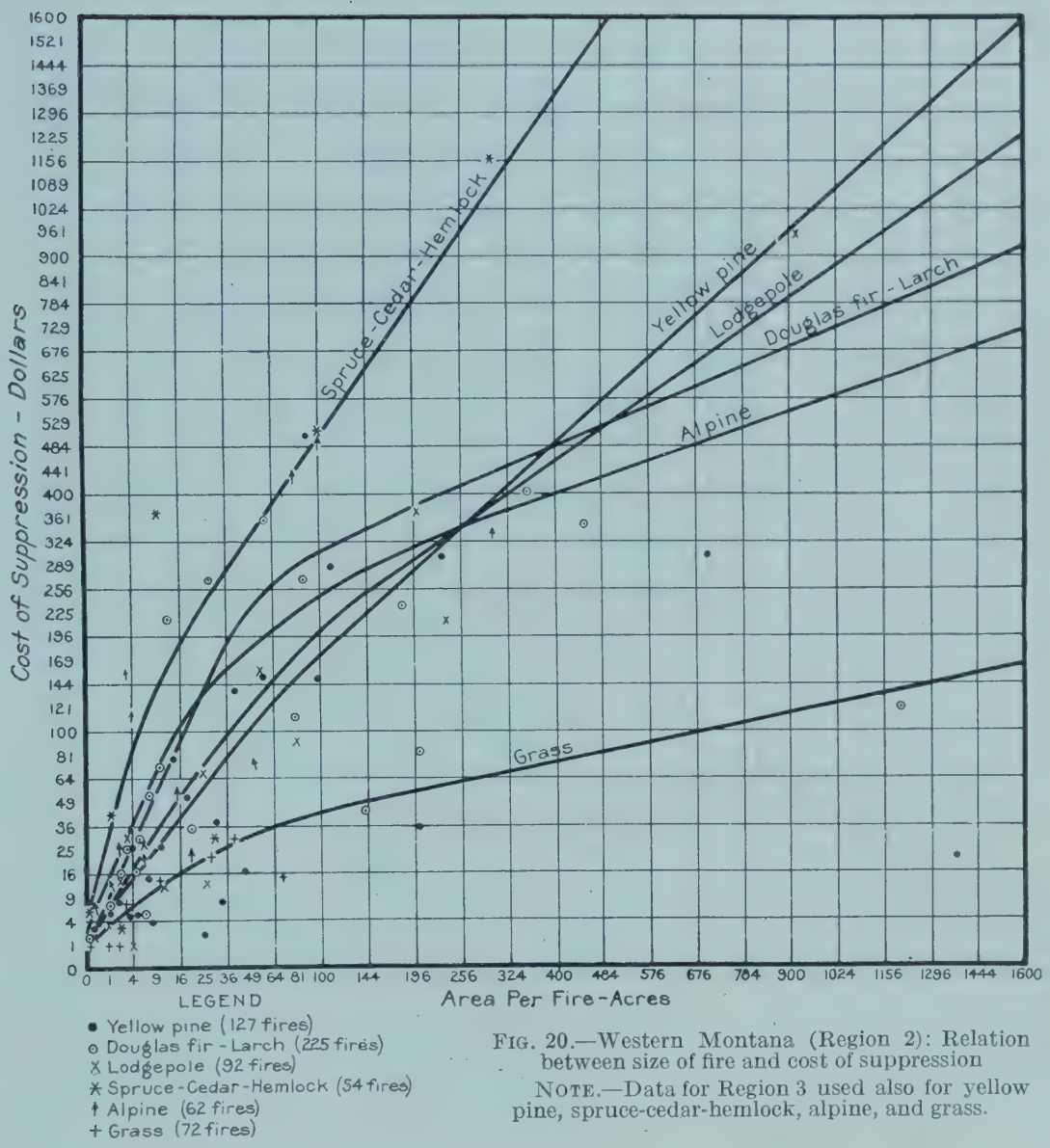


FIG. 20.—Western Montana (Region 2): Relation between size of fire and cost of suppression

NOTE.—Data for Region 3 used also for yellow pine, spruce-cedar-hemlock, alpine, and grass.

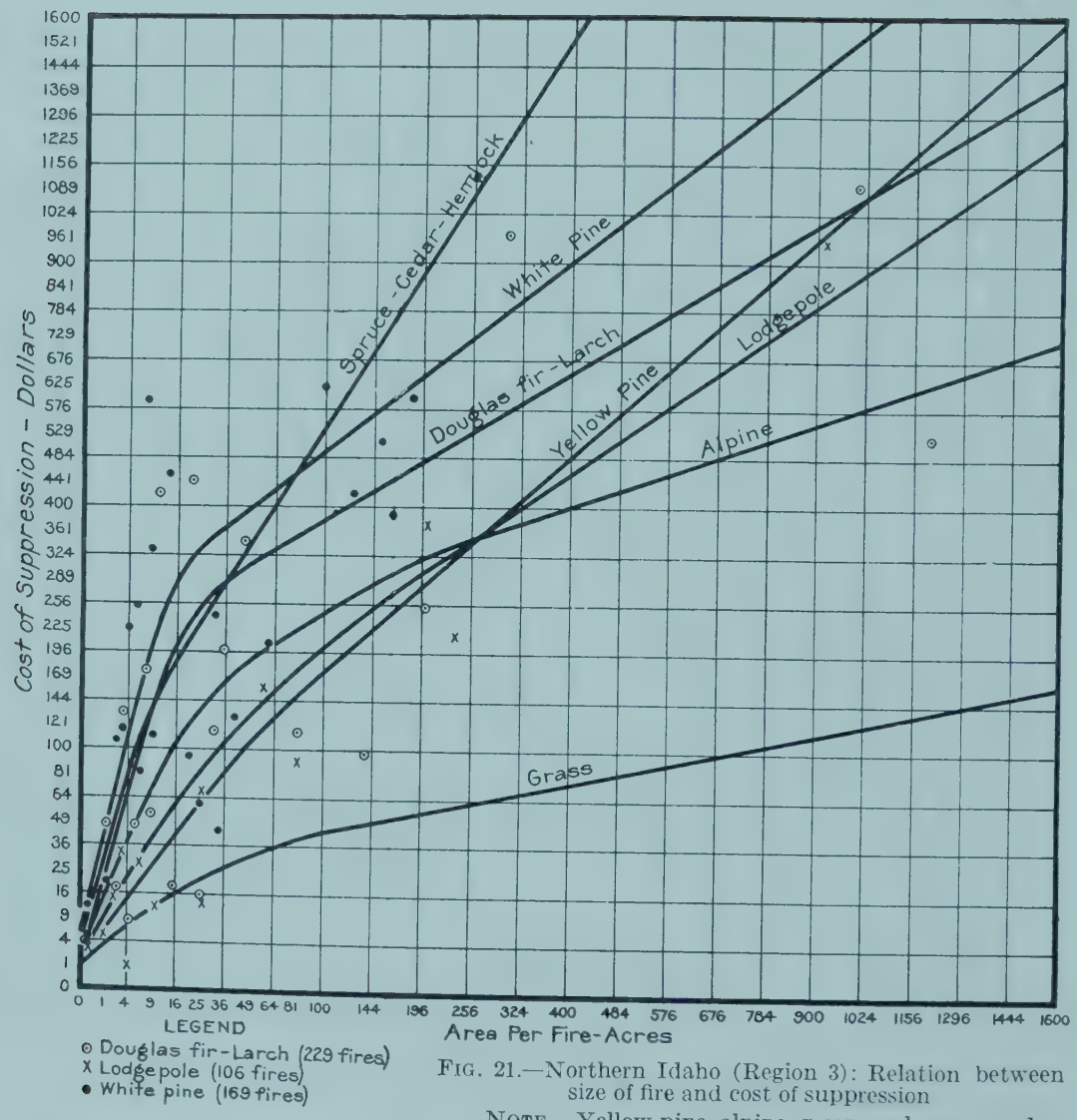


FIG. 21.—Northern Idaho (Region 3): Relation between size of fire and cost of suppression

NOTE.—Yellow pine, alpine, grass, and spruce-cedar-hemlock curves are identical with Western Montana curves. Lodgepole curve based chiefly on Western Montana data.

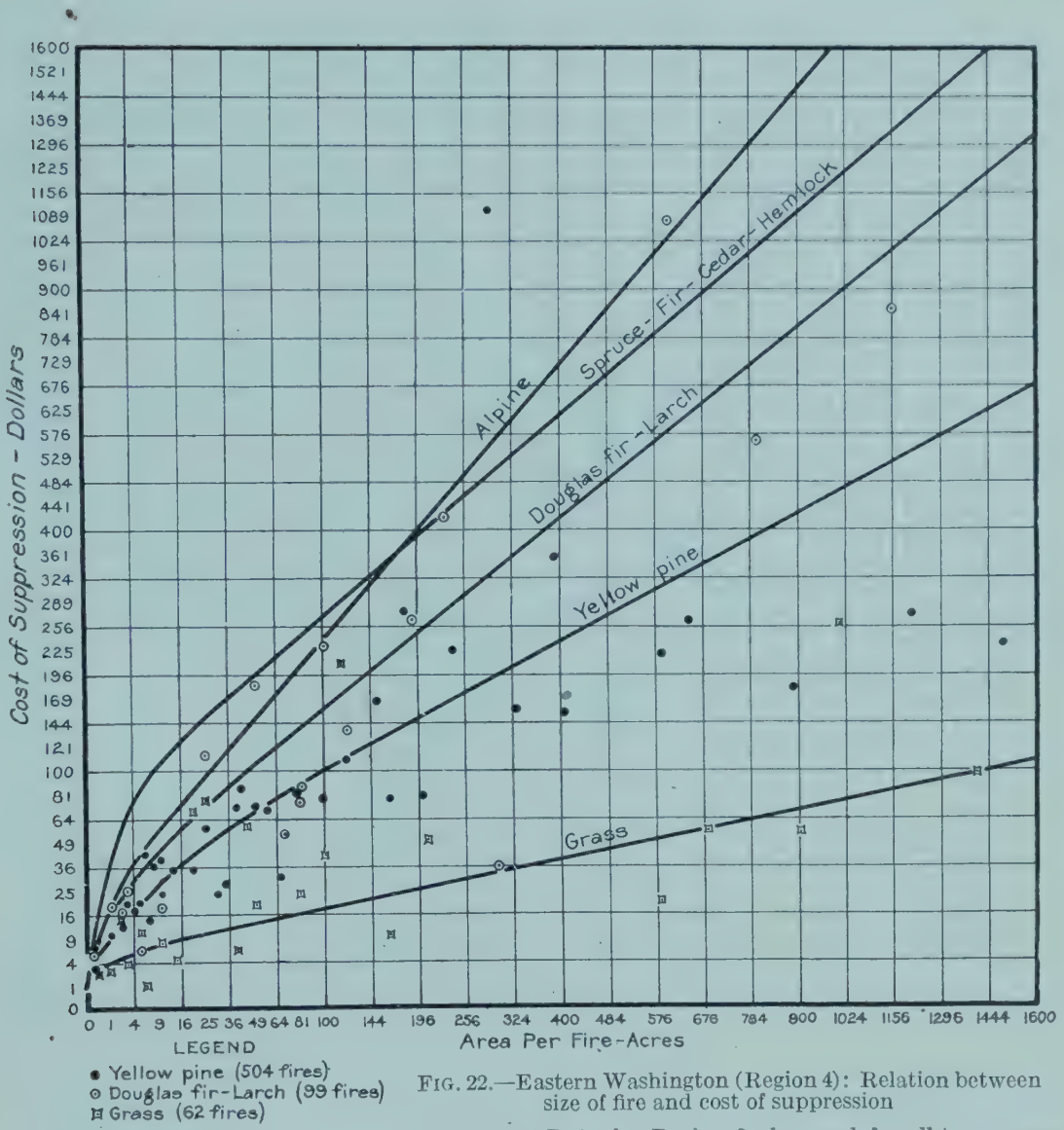


FIG. 22.—Eastern Washington (Region 4): Relation between size of fire and cost of suppression

NOTE.—Data for Region 8 also used for all types except Douglas fir-larch.

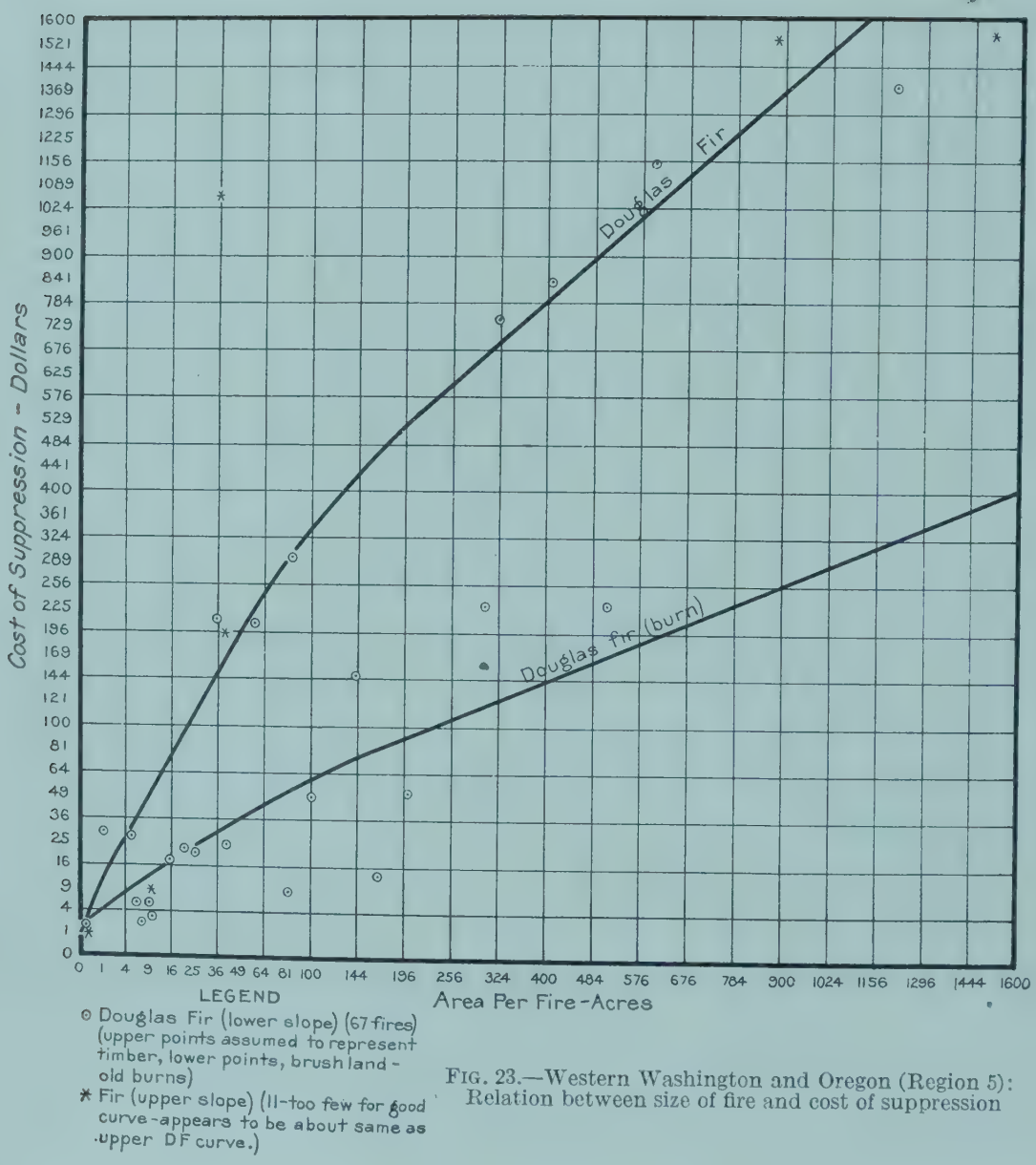


FIG. 23.—Western Washington and Oregon (Region 5): Relation between size of fire and cost of suppression

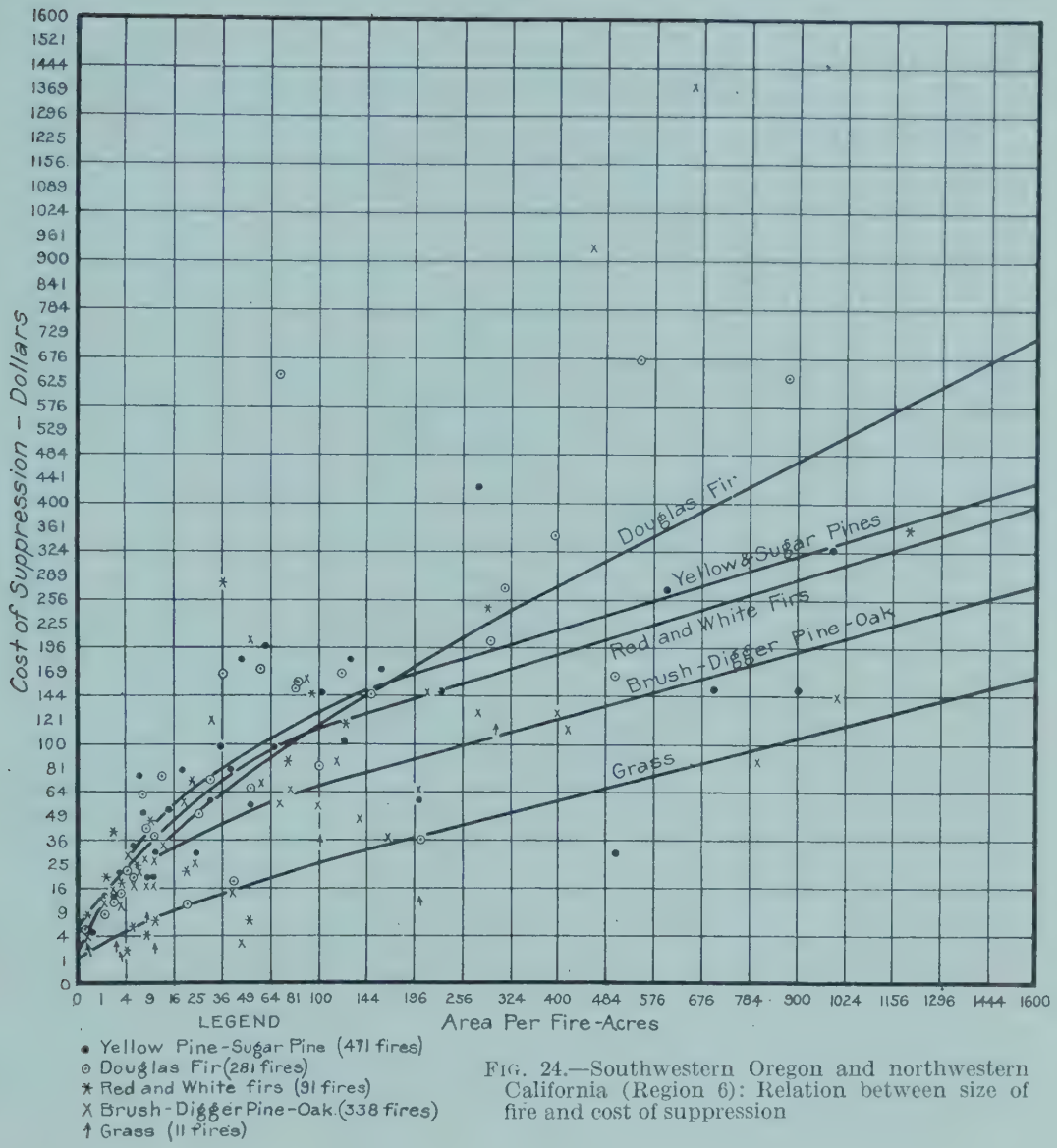


FIG. 24.—Southwestern Oregon and northwestern California (Region 6): Relation between size of fire and cost of suppression

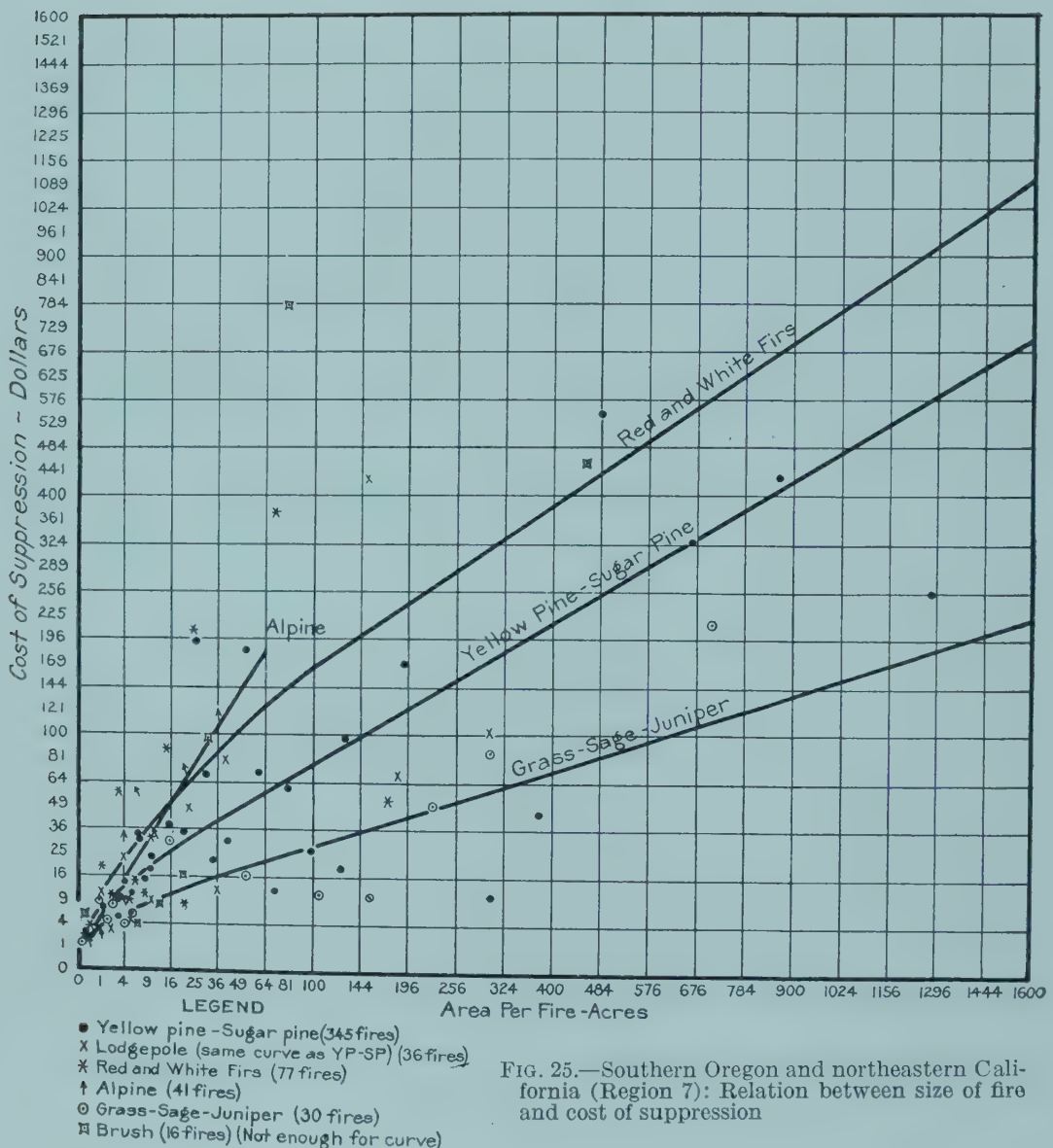


FIG. 25.—Southern Oregon and northeastern California (Region 7): Relation between size of fire and cost of suppression

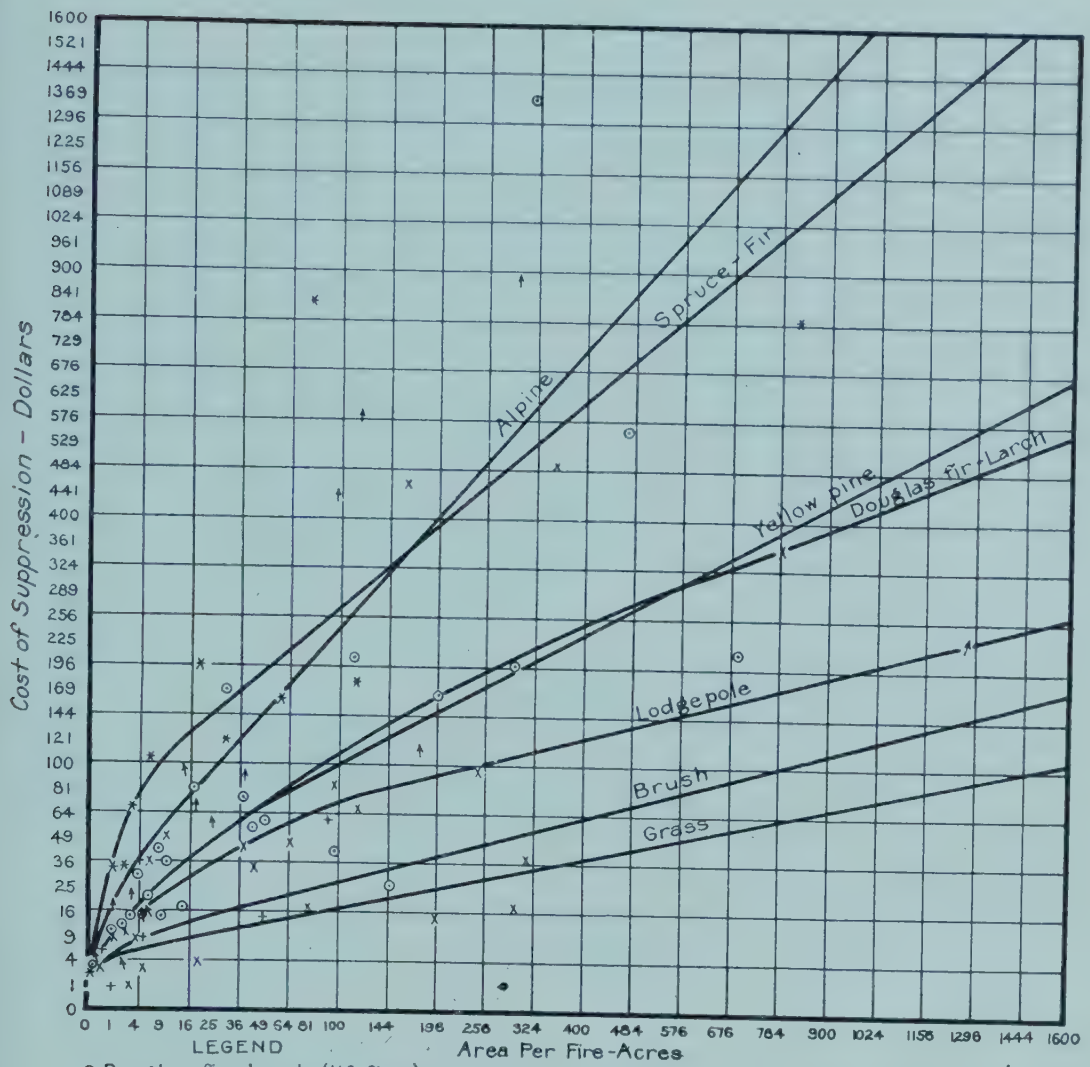


FIG. 26.—Eastern Oregon and southwestern Idaho (Region 8): Relation between size of fire and cost of suppression

NOTE.—Curves for all types except brush and Douglas fir-larch are identical with those for Region 4.

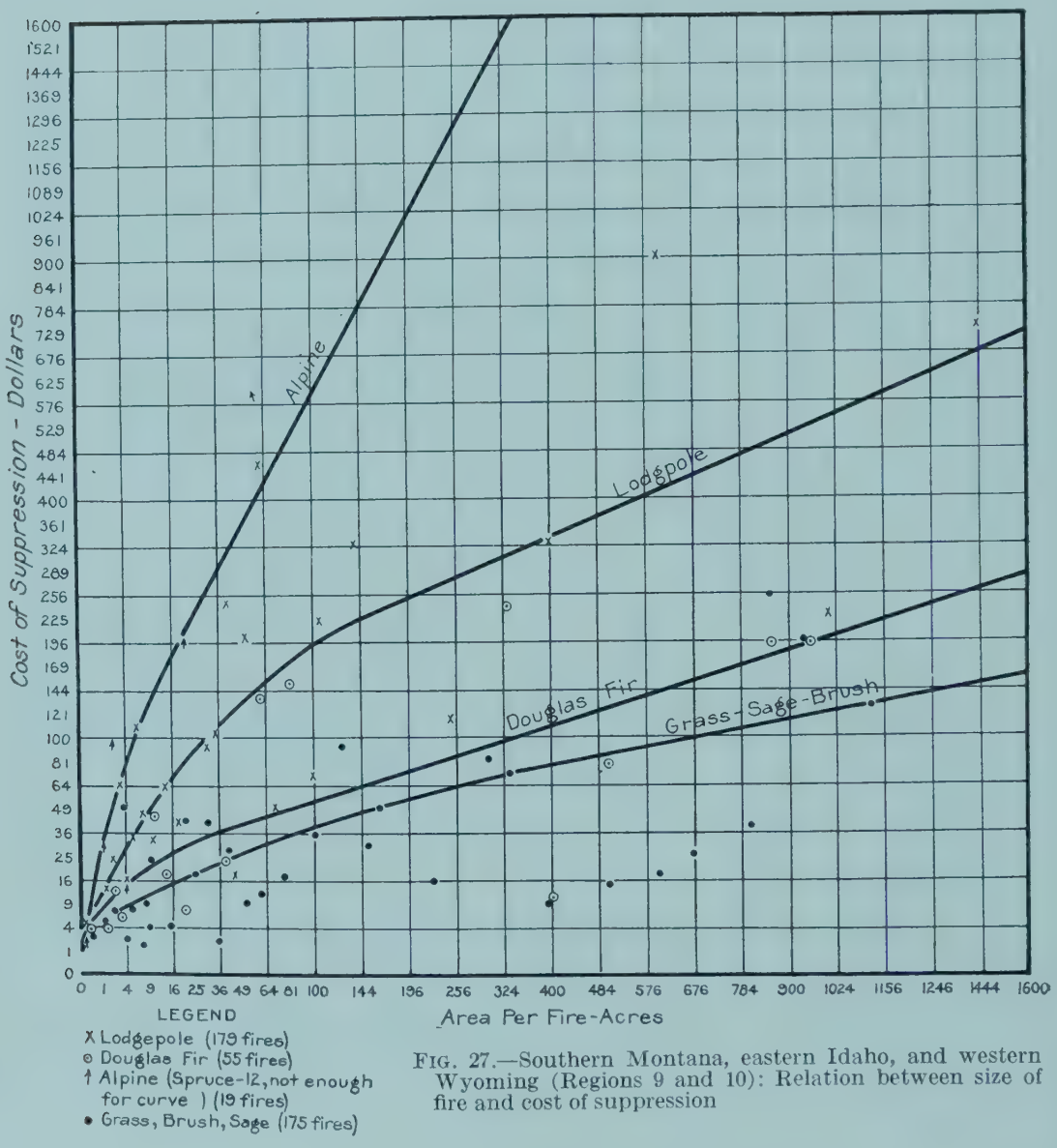


FIG. 27.—Southern Montana, eastern Idaho, and western Wyoming (Regions 9 and 10): Relation between size of fire and cost of suppression

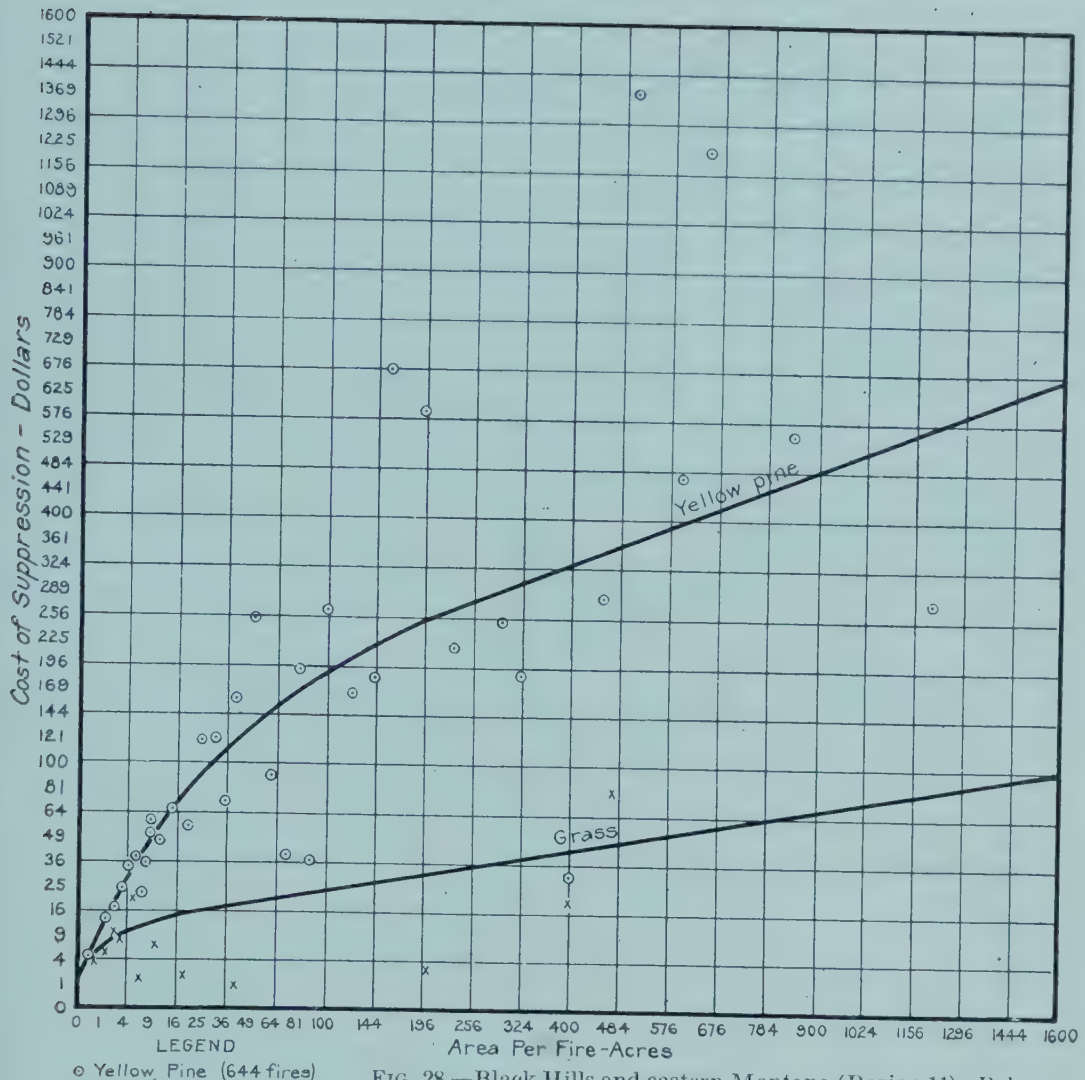


FIG. 28.—Black Hills and eastern Montana (Region 11): Relation between size of fire and cost of suppression

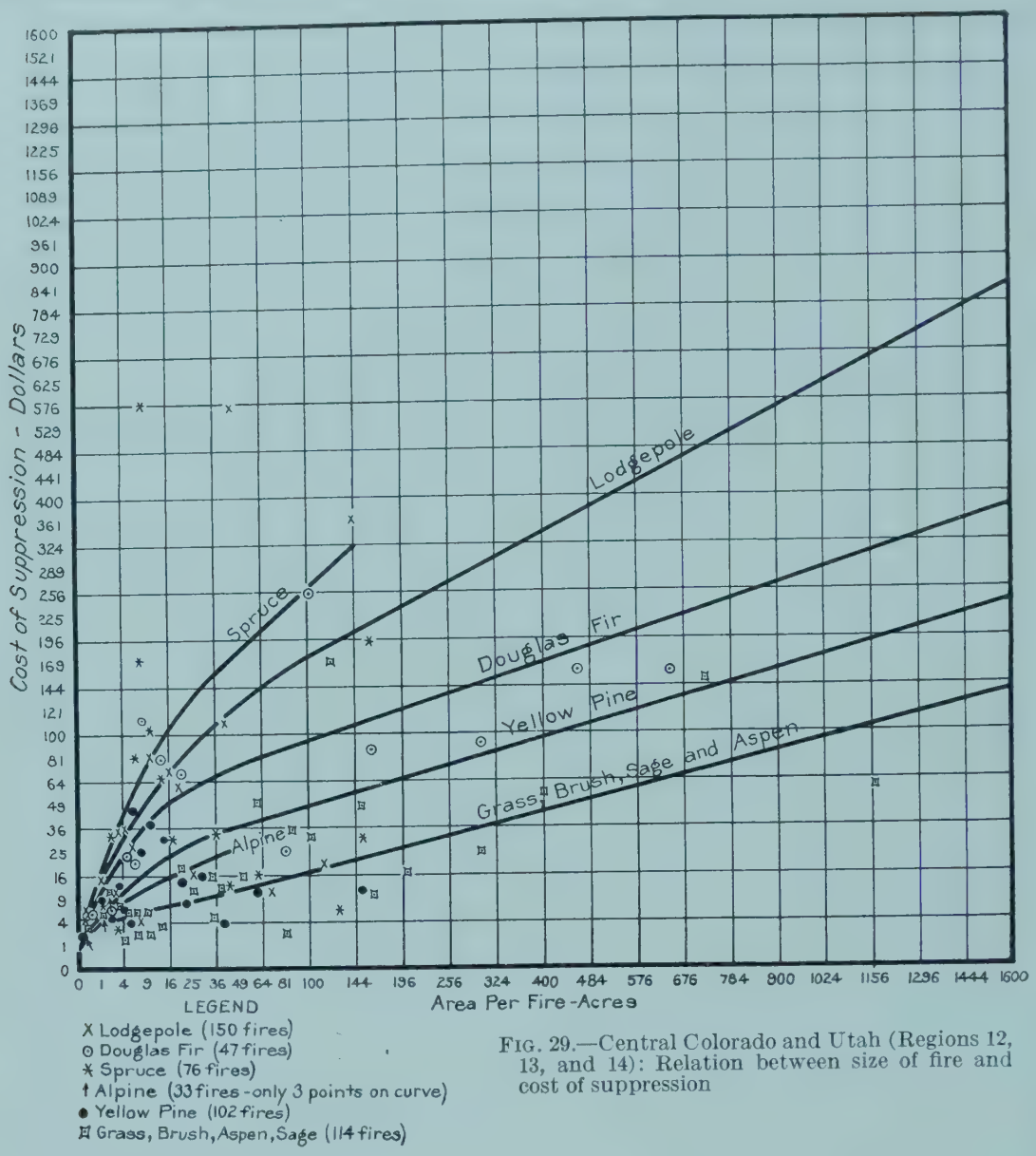


FIG. 29.—Central Colorado and Utah (Regions 12, 13, and 14): Relation between size of fire and cost of suppression

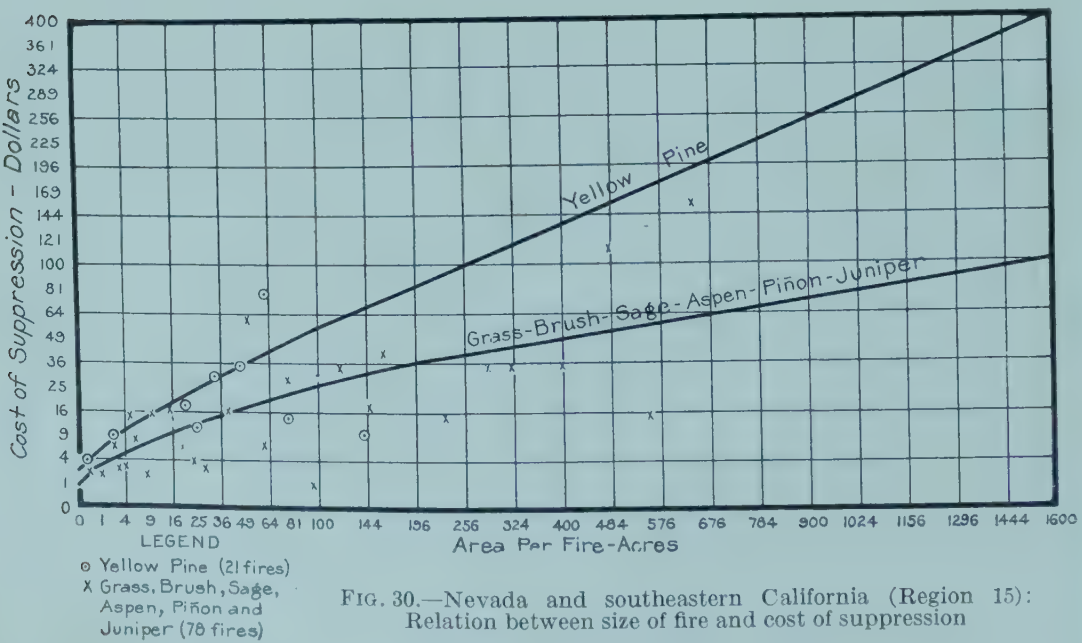


FIG. 30.—Nevada and southeastern California (Region 15): Relation between size of fire and cost of suppression

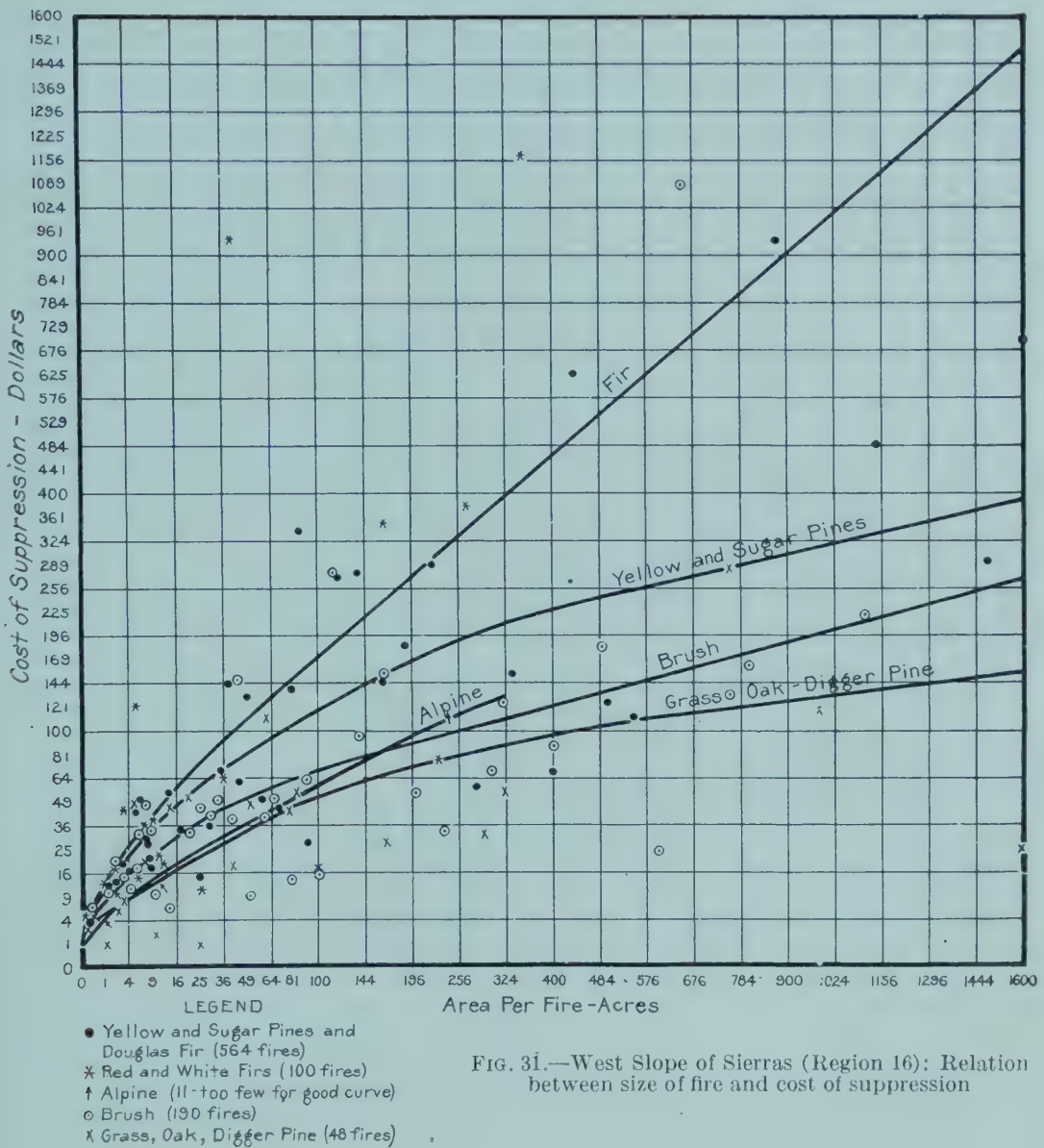


FIG. 31.—West Slope of Sierras (Region 16): Relation between size of fire and cost of suppression

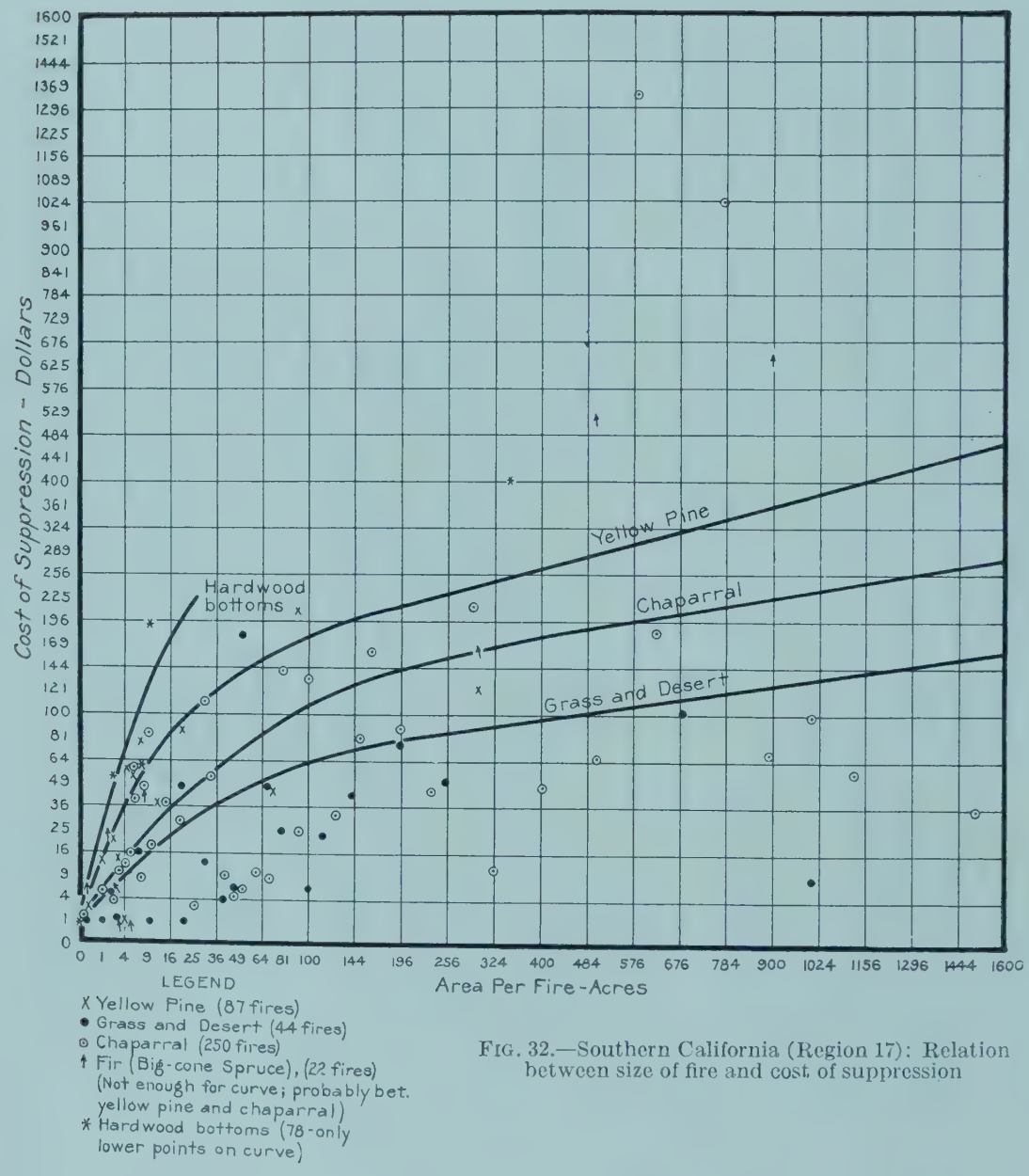


FIG. 32.—Southern California (Region 17): Relation between size of fire and cost of suppression

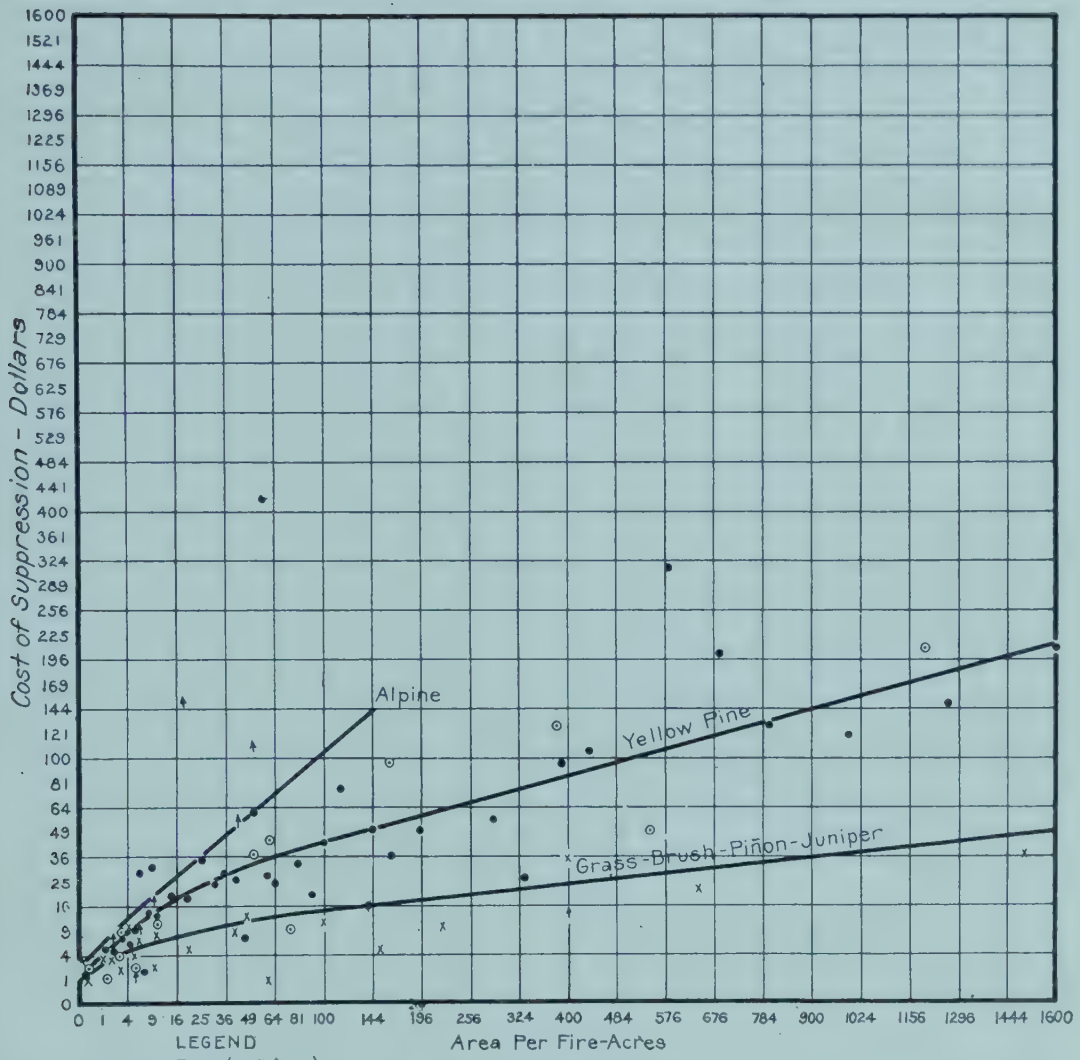


FIG. 33.—Northern Arizona and Western New Mexico (Region 18): Relation between size of fire and cost of suppression

NOTE.—Data for Region 20 included for alpine type.

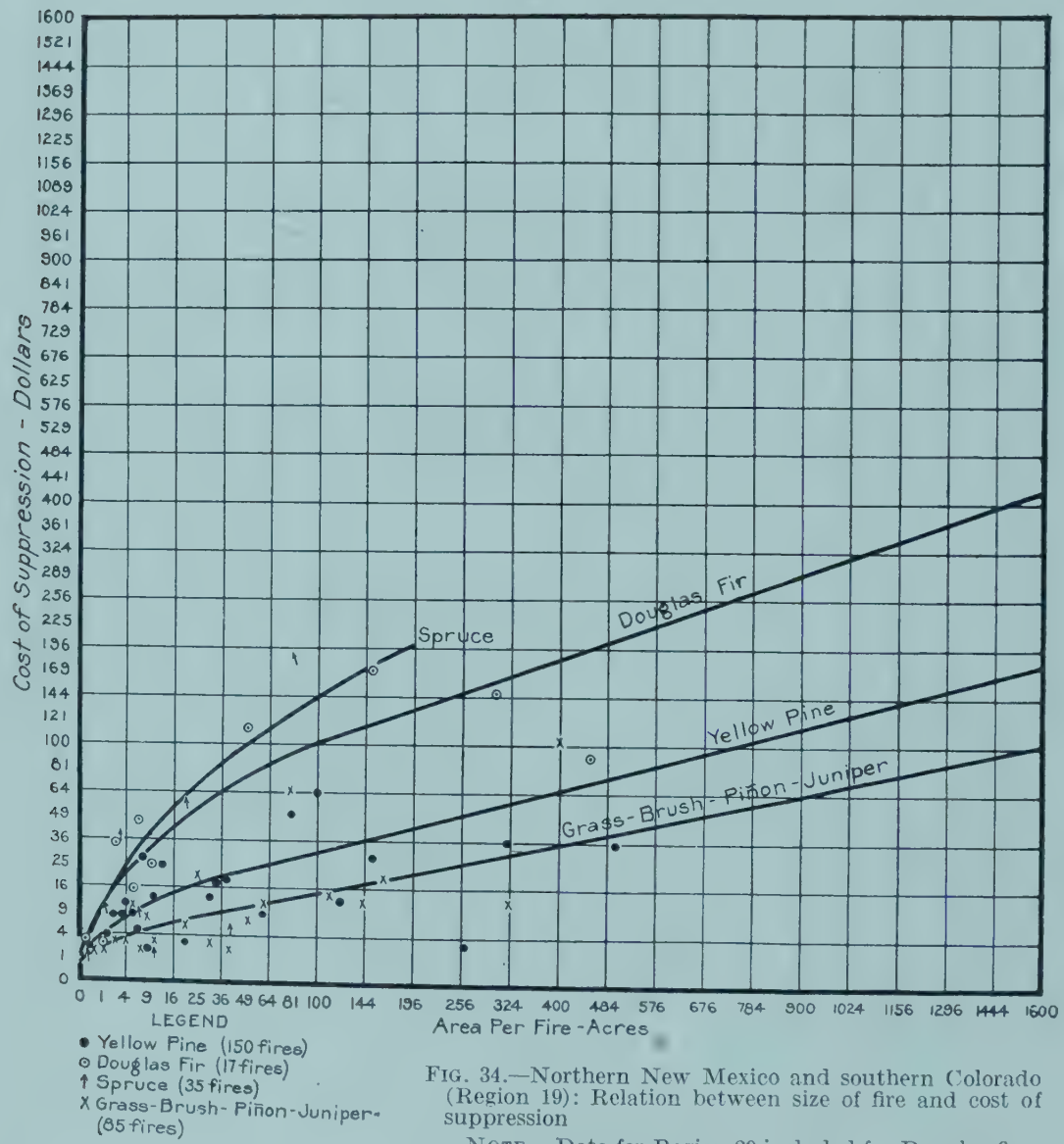


FIG. 34.—Northern New Mexico and southern Colorado (Region 19): Relation between size of fire and cost of suppression

NOTE.—Data for Region 20 included for Douglas fir.

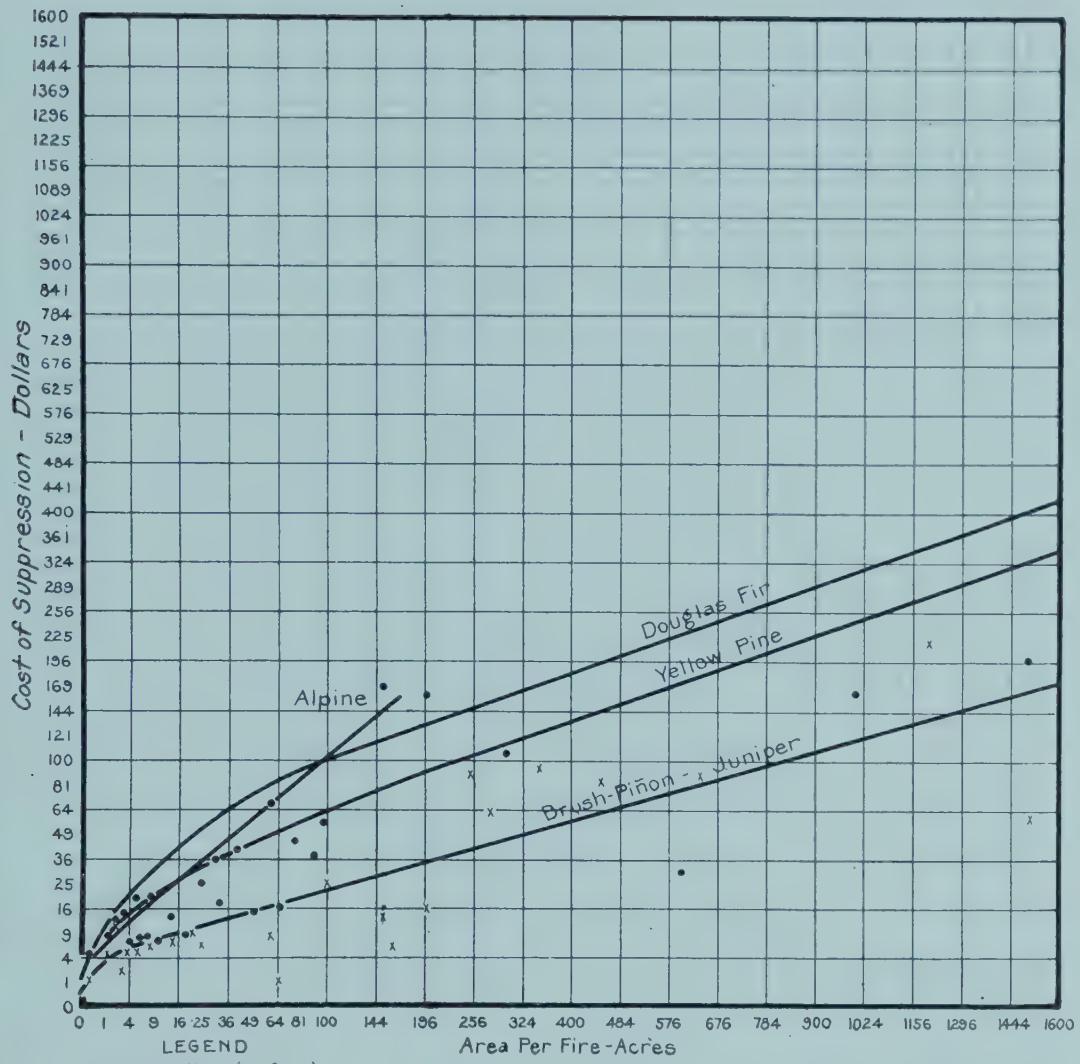


FIG. 35.—Southern Arizona and New Mexico (Region 20): Relation between size of fire and cost of suppression

NOTE.—Curve for Douglas fir identical with that for Region 19; alpine curve same as that for Region 18.

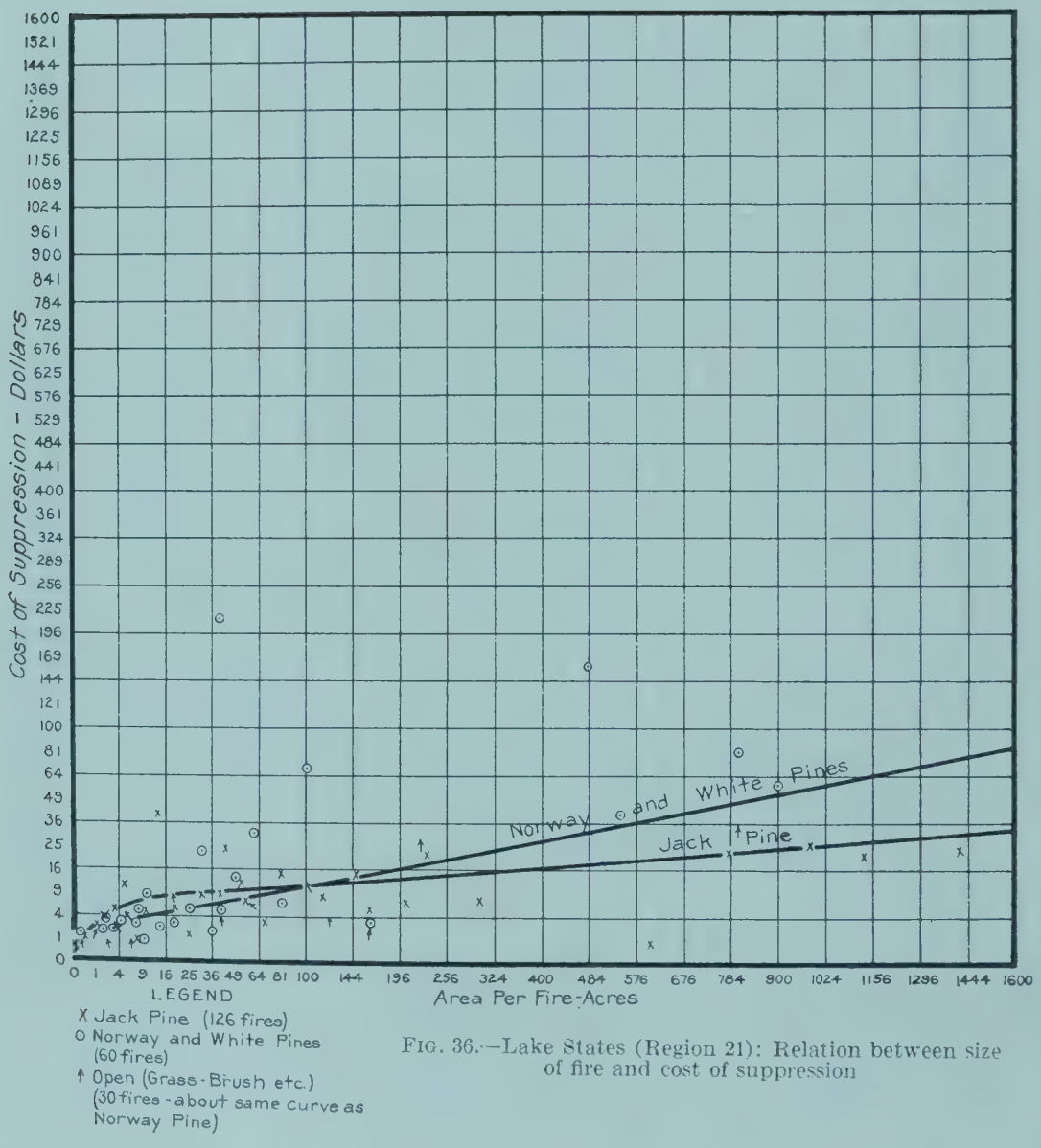


FIG. 36.—Lake States (Region 21): Relation between size of fire and cost of suppression

TABLE VI.—*Average costs of suppressing fires, according to time elapsed between detection and start of suppression work* [a]

REGION 2—WESTERN MONTANA, 1911–1915

Time elapsed (hours)	Forest types						
	Western yellow pine	Douglas fir and larch	Western white pine	Lodgepole pine	Spruce, hemlock, white fir	Subalpine	Open
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	11.00	15.00	42.00	10.00	12.00	11.00	12.00
1	16.00	25.00	69.00	14.00	14.00	15.00	17.00
2	27.00	48.00	92.00	19.00	17.00	19.00	24.00
3	34.00	72.00	110.00	24.00	19.00	21.00	29.00
4	40.00	88.00	130.00	26.00	21.00	22.00	33.00
5	46.00	102.00	155.00	28.00	24.00	24.00	36.00
6	52.00	115.00	175.00	35.00	26.00	26.00	39.00
7	58.00	130.00	200.00	50.00	29.00	27.00	42.00
8	64.00	145.00	230.00	62.00	32.00	29.00	45.00
9	72.00	165.00	250.00	72.00	36.00	35.00	46.00
10	80.00	185.00	270.00	81.00	40.00	58.00	49.00
12	96.00	215.00	295.00	100.00	49.00	88.00	52.00
15	120.00	255.00	325.00	130.00	59.00	110.00	59.00
20	155.00	290.00	360.00	185.00	81.00	135.00	71.00

REGION 3—NORTHERN IDAHO

Time elapsed (hours)	Forest types						
	Western yellow pine	Douglas fir and larch	Western white pine	Lodgepole pine	Spruce, fir, cedar, hemlock	Subalpine	Open
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	11.00	42.00	42.00	13.00	12.00	11.00	12.00
1	16.00	74.00	69.00	17.00	14.00	15.00	17.00
2	27.00	135.00	92.00	23.00	17.00	19.00	24.00
3	34.00	180.00	110.00	28.00	19.00	21.00	29.00
4	40.00	200.00	130.00	30.00	21.00	22.00	33.00
5	46.00	220.00	155.00	33.00	24.00	24.00	36.00
6	52.00	235.00	175.00	40.00	26.00	26.00	39.00
7	58.00	245.00	200.00	58.00	29.00	27.00	42.00
8	64.00	260.00	230.00	72.00	32.00	29.00	45.00
9	72.00	270.00	250.00	81.00	36.00	35.00	46.00
10	80.00	280.00	270.00	88.00	40.00	58.00	49.00
12	96.00	300.00	295.00	110.00	49.00	88.00	52.00
15	120.00	325.00	325.00	135.00	59.00	110.00	59.00
20	155.00	360.00	360.00	185.00	81.00	135.00	71.00

[a] Tables prepared for Regions 1, 5, 7, 9, 10, 11, 15, 18, and 20 are omitted because of the rather inadequate data on which they are based.

TABLE VI.—*Average costs of suppressing fires, according to time elapsed between detection and start of suppression work*—Continued

REGION 4—EASTERN WASHINGTON

Time elapsed (hours)	Forest types						
	Western yellow pine	Douglas fir and larch	Lodgepole pine	Spruce, cedar, white fir, hemlock	Subalpine	Open	
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	
½	15.00	19.00	13.00	25.00	11.00	16.00	
1	20.00	25.00	14.00	36.00	15.00	19.00	
2	29.00	30.00	15.00	48.00	20.00	22.00	
3	38.00	36.00	18.00	55.00	25.00	26.00	
4	49.00	44.00	24.00	64.00	26.00	29.00	
5	59.00	58.00	27.00	68.00	27.00	33.00	
6	69.00	76.00	30.00	73.00	28.00	37.00	
7	79.00	105.00	33.00	78.00	29.00	42.00	
8	90.00	135.00	35.00	83.00	30.00	48.00	
9	100.00	180.00	37.00	88.00	31.00	53.00	
10	115.00	220.00	41.00	94.00	32.00	58.00	
12	135.00	325.00	46.00	100.00	42.00	72.00	
15	175.00	510.00	54.00	110.00	62.00	92.00	
20	250.00	925.00	69.00	125.00	98.00	130.00	

REGION 6—SOUTHERN OREGON AND NORTHERN CALIFORNIA COAST RANGES

Time elapsed (hours)	Forest types						
	Western yellow and sugar pine	Douglas fir	Red and white fir	Subalpine [a]	Digger pine–oak	Brush	Grass
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	27.00	12.00	12.00	8.00	66.00	35.00	24.00
1	40.00	15.00	13.00	8.25	76.00	44.00	30.00
2	56.00	20.00	14.00	8.50	85.00	53.00	35.00
3	67.00	34.00	16.00	8.75	92.00	64.00	40.00
4	78.00	44.00	17.00	9.00	100.00	70.00	45.00
5	86.00	53.00	19.00	9.25	108.00	76.00	50.00
6	94.00	61.00	24.00	9.50	117.00	81.00	56.00
7	100.00	67.00	31.00	9.75	128.00	85.00	62.00
8	108.00	73.00	35.00	10.00	135.00	90.00	67.00
9	115.00	77.00	38.00	10.50	145.00	96.00	74.00
10	120.00	83.00	40.00	11.00	155.00	100.00	81.00
12	130.00	92.00	44.00	11.50	175.00	110.00	96.00
15	145.00	110.00	48.00	12.00	210.00	130.00	120.00
20	170.00	130.00	60.00	16.00	280.00	160.00	165.00

[a] Costs for subalpine are based on red fir curve.

TABLE VI.—*Average costs of suppressing fires, according to time elapsed between detection and start of suppression work*—Continued

REGION 8—EASTERN OREGON AND SOUTHWESTERN IDAHO

Time elapsed (hours)	Forest types						
	Western yellow pine	Douglas fir and larch	Lodgepole pine	Spruce and fir	Subalpine	Brush	Grass and sage
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	15.00	10.00	13.00	25.00	11.00	24.00	16.00
1	20.00	14.00	14.00	26.00	15.00	29.00	19.00
2	29.00	18.00	15.00	48.00	20.00	34.00	22.00
3	38.00	22.00	18.00	55.00	25.00	40.00	26.00
4	49.00	28.00	24.00	64.00	26.00	46.00	29.00
5	59.00	37.00	27.00	68.00	27.00	53.00	33.00
6	69.00	49.00	30.00	73.00	28.00	61.00	37.00
7	79.00	70.00	33.00	78.00	29.00	67.00	42.00
8	90.00	100.00	35.00	83.00	30.00	76.00	48.00
9	100.00	120.00	37.00	88.00	31.00	85.00	53.00
10	115.00	145.00	41.00	94.00	32.00	95.00	58.00
12	135.00	200.00	46.00	100.00	42.00	115.00	72.00
15	175.00	285.00	54.00	110.00	62.00	145.00	92.00
20	250.00	430.00	69.00	125.00	98.00	210.00	130.00

REGION 12—EASTERN COLORADO; REGION 13—NORTHWESTERN COLORADO AND SOUTHERN WYOMING; REGION 14—WASATCH AND UINTA RANGES (UTAH)[a]

Time elapsed (hours)	Forest types						
	Western yellow pine	Douglas fir	Lodgepole pine	Engelmann spruce	Subalpine	Woodland and aspen brush	Grass and sage
	Dolls.	*Dolls.*	*Dolls.*	*Dolls*	*Dolls.*	*Dolls.*	*Dolls.*
½	9.00	19.00	20.00	22.00	4.00	10.00	7.00
1	11.00	24.00	30.00	28.00	6.00	14.00	8.50
2	13.00	36.00	50.00	36.00	8.00	18.00	10.00
3	15.00	45.00	66.00	45.00	11.00	20.00	13.00
4	16.00	50.00	83.00	50.00	11.50	22.00	15.00
5	18.00	56.00	100.00	56.00	12.00	24.00	18.00
6	20.00	61.00	115.00	62.00	13.00	26.00	20.00
7	24.00	66.00	130.00	69.00	14.00	28.00	24.00
8	28.00	71.00	145.00	74.00	14.50	30.00	26.00
9	32.00	75.00	160.00	81.00	15.00	32.00	30.00
10	35.00	80.00	170.00	90.00	15.50	35.00	34.00
12	41.00	85.00	195.00	100.00	16.00	40.00	42.00
15	50.00	100.00	230.00	120.00	18.00	49.00	56.00
20	67.00	120.00	300.00	150.00	21.00	64.00	83.00

[a] These three regions combined in order to afford better basis for curves. They are fairly similar.

TABLE VI.—*Average costs of suppressing fires, according to time elapsed between detection and start of suppression work*—Continued

REGION 16—WEST SLOPE OF SIERRAS

Time elapsed (hours)	Forest types					
	Western yellow and sugar pines and Douglas fir	Red and white firs	Subalpine	Lodgepole[a] and knobcone pines	Digger pine–oak and grass	Brushfields
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	23.00	10.00	1.00	23.00	53.00	60.00
1	36.00	15.00	1.50	36.00	61.00	72.00
2	52.00	21.00	1.75	52.00	68.00	81.00
3	64.00	22.00	2.00	64.00	74.00	88.00
4	72.00	23.00	2.00	72.00	81.00	94.00
5	81.00	24.00	2.00	81.00	87.00	99.00
6	86.00	25.00	2.00	86.00	93.00	104.00
7	90.00	26.00	2.00	90.00	98.00	109.00
8	94.00	26.50	2.00	94.00	104.00	115.00
9	100.00	27.00	2.25	100.00	108.00	120.00
10	105.00	28.00	2.50	105.00	112.00	125.00
12	110.00	29.50	3.00	110.00	120.00	140.00
15	120.00	31.00	3.50	120.00	130.00	155.00
20	145.00	34.00	6.00	145.00	155.00	190.00

[a] Because of insufficient data for lodgepole type, figures for the yellow pine, sugar pine, and incense cedar type were used.

REGION 17—SOUTHERN CALIFORNIA

Time elapsed (hours)	Forest types					
	Western yellow and Jeffrey pine	Fir[a] and pine slopes	Subalpine[b]	Chaparral	Hardwood bottoms	Grass and sage
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	28.00	24.00	1.00	145.00	32.00	50.00
1	45.00	45.00	1.50	160.00	55.00	62.00
2	77.00	79.00	1.75	175.00	87.00	70.00
3	102.00	100.00	2.00	190.00	110.00	77.00
4	119.00	115.00	2.00	200.00	125.00	85.00
5	132.00	130.00	2.00	210.00	135.00	90.00
6	145.00	140.00	2.00	220.00	140.00	95.00
7	155.00	150.00	2.00	230.00	145.00	100.00
8	165.00	160.00	2.00	240.00	150.00	105.00
9	170.00	170.00	2.25	250.00	155.00	110.00
10	180.00	180.00	2.50	260.00	165.00	120.00
12	190.00	190.00	3.00	280.00	175.00	130.00
15	200.00	210.00	3.50	315.00	190.00	150.00
20	230.00	245.00	6.00	380.00	215.00	190.00

[a] Based on area from fir slope curve, and costs from chaparral curve.

[b] Based on figures for Region 16 (Sierras).

TABLE VI.—*Average costs of suppressing fires, according to time elapsed between detection and start of suppression work*—Continued

REGION 19—SOUTHWESTERN COLORADO AND NORTHERN NEW MEXICO

Time elapsed (hours)	Forest types						
	Western yellow pine	Douglas fir mixed	Spruce and subalpine	Lodgepole pine [a]	Pinon-juniper	Brush	Grass
	Dollars	*Dollars*	*Dollars*	*Dollars*	*Dollars*	*Dollars*	*Dollars*
½	7.50	13.00	25.00	20.00	1.75	5.00	7.00
1	9.50	18.00	34.00	30.00	2.00	6.00	9.00
2	14.00	28.00	47.00	50.00	2.15	7.00	12.00
3	17.00	40.00	53.00	66.00	2.30	8.00	16.00
4	20.00	50.00	59.00	83.00	2.45	9.00	20.00
5	23.00	61.00	65.00	100.00	2.60	10.00	25.00
6	26.00	72.00	69.00	115.00	2.80	11.00	30.00
7	29.00	81.00	72.00	130.00	3.00	12.00	35.00
8	32.50	90.00	77.00	145.00	3.25	13.00	41.00
9	36.00	99.00	81.00	160.00	3.50	14.00	46.00
10	39.50	106.00	85.00	170.00	3.75	15.00	54.00
12	47.00	120.00	94.00	195.00	4.00	17.00	67.00
15	59.00	145.00	104.00	230.00	4.25	20.00	94.00
20	83.00	190.00	123.00	300.00	5.00	27.00	145.00

[a] For lodgepole pine, figures for Region 13 were used.

REGION 21—LAKE STATES

Time elapsed (hours)	Forest types				
	Eastern white and red pine (green)	White and red pine (slash, etc.)	Jack pine (mostly slash)	Other timber (green) [a]	Open
	Dollars	*Dollars*	*Dollars*	*Dollars*	*Dollars*
½	3.00	9.50	18.00	3.00	5.00
1	4.00	11.50	21.00	4.00	6.00
2	4.50	16.00	23.00	4.50	7.00
3	5.00	21.00	27.00	5.00	9.00
4	5.50	27.00	31.00	5.50	10.00
5	6.00	34.00	35.00	6.00	11.50
6	6.25	41.50	39.00	6.25	13.50
7	6.75	49.00	44.00	6.75	15.00
8	7.25	58.00	48.00	7.25	17.00
9	7.75	69.00	51.00	7.75	18.50
10	8.00	79.00	55.00	8.00	21.00
12	9.00	100.00	66.00	9.00	25.00
15	10.50	145.00	83.00	10.50	32.50
20	13.00	235.00	115.00	13.00	47.00

[a] Because of insufficient data for fires in other timber types (spruce, balsam, tamarack, hardwoods), the same figures were used as for white and Norway pine green.

AMOUNT OF DAMAGE DONE BY FIRES

Damage varies directly with area burned, and therefore may be expressed on an acre basis. The question of damage is the most difficult part of the whole problem, for several reasons. In the first place, no satisfactory method has yet been devised for putting money value on the less tangible elements of value present in a forest. Even the value of merchantable timber can be determined with a reasonable degree of accuracy only where such timber is so located as to be immediately saleable, and the value of the same timber varies more or less from year to year. In case of young growth, a number of complications enter which make it almost impossible to value it on a scientific basis, or rather, to determine the monetary loss in case of its destruction or injury by fire. The fire reports, in the majority of cases, entirely neglect to evaluate the damage to young growth, or estimate it so crudely and inconsistently that the figures are worthless. Damage to forage is ignored, not only because of the extreme paucity of data, but also because the existing data indicate that such damage is less than the probable error in estimating damage to timber. In studying the records of individual fires, then, it was decided to ignore the estimates of damage expressed in dollars, and to consider only the data as to quantity of damage expressed in board feet of timber and acres of reproduction. The fire records are particularly incomplete on these points, especially in regard to reproduction destroyed, but they represent all the information that can be readily obtained. For those fires within each type and subregion for which data were available regarding amount of damage, the average damage per acre burned over was ascertained. The figures given in Table VII are in each case averages for all burns in the given types, regardless of age class or density of stand, so should not be taken as indication of the amount of damage in mature well-stocked stands. For this reason the average amount of damage given for the mixed pine type of the Sierras, for instance, is less than the amount indicated by studies made in mature stands. It is important to know the relation between the quantities of timber and young growth present on the burned area before the fire and the amount destroyed—in other words, what the ratio of destruction is. Data on this point are even more fragmentary than those on the total amount of damage, but such as are available were compiled.

TABLE VII.—*Average amount of damage done by fires in different types and regions, 1911–1915*[a]

Type	Region	Timber destroyed		Young growth killed	
		Amount per acre	Percentage of stand	Percentage of burned area	Percentage of original stand
		Bd. ft.			
Yellow pine, including western yellow pine, sugar pine, incense cedar, and white fir mixture of California.	2, 3	695	14.9	56	83
	4	420	14.8	48	77
	6	860	12.4	46	63
	7	820	7.0	36	66
	8	950	8.4	70	93
	11	500	40.0	20	68
	12, 14	290	39.6	21	48
	15	385	8.3	44	88
	16	630	7.6	27	50
	17	560	6.7	44	58
	18, 19	100	4.4	26	47
	20	200	18.6	52	77
Douglas fir, including western larch and other mixtures.	1, 9, 10	775	79.6	56	67
	2	855	30.1	59	89
	3	1,290	47.0	87	97
	4	3,580	44.4	49	95
	5	1,630	45.3	64	82
	6	425	11.9	65	91
	8	1,895	70.1	29	56
	12, 13, 14, 15	195	25.5	33	92
	17	190	12.1	38	60
	18, 19, 20	530	23.1	23	32
Lodgepole pine	1, 9, 10, 15	1,180	80.8	48	83
	2, 3, 4, 5	320	39.4	83	84
	7, 8	585	65.4	36	81
	12, 13, 14, 19	2,090	96.8	72	99
Spruce and firs, including western hemlock and western red cedar except west of Cascades. (For Arizona, Colorado, Utah, and Nevada, the figures are for this type and subalpine combined).	1, 9, 10	1,685	99.9	16	100
	2, 3, 4	7,850	82.8	64	96
	5	5,000	100.0	48	100
	6	455	13.5	67	92
	7, 16	850	10.6	48	76
	8	1,445	19.5	28	84
	12, 13, 14, 15, 19	1,260	73.9	48	97
	18, 20	55	8.4	54	72
Western white pine	2, 3	3,860	84.7	33	96
Subalpine (figures doubtless include some merchantable spruce and fir stands in the Rocky Mountain Region.	1, 9, 10	3,985	80.7	99	100
	2, 3, 4, 5	1,670	65.1	28	66
	6, 7, 16, 17	130	12.1	19	37
	8	765	72.7	70	90
	12, 13, 14, 15, 19	1,260	73.9	48	97
	18, 20	55	8.4	54	72
Woodland.[b] Includes pinon-juniper and digger pine-oak. (Converted on basis 2 cords=1,000 bd. ft.)	6, 16	100	14.3	24	93
	7, 14, 15	305	39.3	6	70
	18, 19, 20	510	55.3	39	87
Brushland.[b] Includes woodland and aspen in some cases.	6, 7, 8, 16	110	9.0	8	77
	9, 10, 12, 13, 14, 15	160	No data	4	No data
	17	70	36.3	2	89
	18, 19, 20	10	.3	0	100
Grass and sage.[b] Includes brushland in regions where no separate figure is given for brush.	1, 9, 10	55	35.4	19	81
	2, 3, 4, 8	60	13.3	4	61
	6, 7, 16	10	1.2	0.4	12
	11	5	8.8	7	15
	12, 13, 14	75	79.3	1	100
	15	270	22.7	7	100
	17, 18, 19, 20	70	13.5	2	89
Hardwood	17	90	5.9	No data	
Eastern white pine–red (Norway) pine	21	60	3.0	18	99
Jack pine[c]	21	205	75.5	24	77

[a] Based on data in individual fire reports.

[b] Board foot figures for losses in woodland and open types are based on insufficient data and often probably too high. Percentages are based on fewer data than are board foot values.

[c] No data for other types in Lake States.

TIMBER VALUES.—For valuing damages in terms of money, it seems advisable to use general figures where averages are concerned, and not to attempt too great detail in the process. It is considered that practically the same loss is suffered in case of destruction of a given quantity of a given species in a given region, whether the particular stand destroyed is accessible to present logging operations, or whether it is less accessible and consequently of less immediate market value. To put a low estimate on the value of more remote timber would result in low estimates of liability, and therefore in less intensive protection and possible large losses of timber. This would defeat one of the important objects of the national forests, viz, to preserve the less accessible timber until it is needed by the country. Moreover, no one can tell what such stumpage may be worth by the time it becomes marketable. If a stand of timber is destroyed the loss is not merely the value of the timber as such, but includes also its value as part of the productive forest capital. The destruction of a million board feet, wherever located, reduces the forest capital and therefore the potential annual yield of the region.

For the purposes of this study, therefore, arbitrary stumpage values were taken, based largely on appraised or bid prices in large timber sales during the past several years, and supplemented by arbitrary estimates where such basis was lacking (Table VIII). In order to apply these figures in estimating damage in different forest types, which usually contain a mixture of species, composite values by types were set, based on assumed proportions of the different species in the mixture.

VALUE OF YOUNG GROWTH.—The problem of valuing young growth is a very complex one, and can be solved satisfactorily only after a great deal of intensive silvicultural research. Expectation values are purely theoretical, and basis for estimating them is lacking, since our knowledge of yields, rotations, costs of management, and even methods of management, is still almost nil. Cost values, according to standard formulae, based on any costs to which large-scale reforestation operations may be reduced, will in very many, perhaps most, cases give greater values for young growth than the present values of fully stocked stands of mature timber on the same sites.

Except for a few types and regions, reforestation costs fixed on the basis of past and present experience are far too high, and probably do not represent at all what the costs will be when

TABLE VIII.—*Basic stumpage values used for estimating damage done to merchantable timber, by species and types*

Species	Values [a]
Western yellow pine	$2 (2), $2.25 (3), $2.50 (4, 12, 14, 15, 18, 19), $2.75 (7, 8), $3 (6, 11, 16, 17, 20).
Sugar pine	$3.50 (6, 7), $4 (16). Norway pine, $4.50 (21).
White pine	Western $3 (2), $3.50 (4), $4 (3). Eastern, $5.50 (21).
Douglas fir	$1 (3), $1.25 (4, 6, 7, 8), $1.50 (1, 2, 12, 13, 16, 17), $1.75 (5, 9), $2 (10, 14, 15), $2.25 (18, 19, 20).
Western larch	$1.25 (3, 4, 8), $1.50 (2). Eastern larch, $2 (21).
Firs (Abies species)	$0.50 (1, 6, 7, 8, 9), $0.75 (2, 4, 5), $1 (3, 10, 12, 13), $1.25 (16), $1.50 (14, 15), $2 (19, 21), $2.25 (18, 20).
Spruce	$1 (8), $1.50 (1, 2, 5, 9), $1.75 (3, 4, 6, 12), $2 (10, 12, 14, 21), $2.25 (18, 19, 20).
Lodgepole pine	$1.50 (2), $1.75 (1, 9, 12), $2 (3, 4, 7, 8, 10, 13, 14, 15), $3 (6). Jack pine, $2 (21).
Western hemlock	$0.50 (5), $0.75 (4), $1 (3).
Cedar (incense and red)	$0.75 (6, IC), $1 (7, IC, 3, 4, RC), $1.25 (16, IC), $1.50 (2, RC), $2 (5, RC).
Aspen	$1 (12, 13, 14, 15).
Pinon-juniper, oak, etc. [b]	$0.25 (6, 7, 8), $0.30 (12), $0.40 (18, 19), $0.50 (14, 15, 16, 17, 20).

Type	Values [a]
Yellow pine, including sugar pine, etc.	$2 (2), $2.20 (12), $2.25 (3), $2.40 (8), $2.50 (4, 14, 15, 17, 18, 19), $2.60 (6), $2.70 (7), $3 (11, 16, 20).
Douglas fir, and larch—fir.	$1.40 (6, 8), $1.50 (1, 3, 4, 5, 12, 13), $1.75 (2, 9), $2 (10, 14, 15), $2.50 (18, 19, 20).
Lodgepole pine	$1.50 (2, 16), $1.60 (8, 9), $1.75 (1, 4, 7, 12), $2 (3, 6, 10, 13, 14, 15, 19). Jack pine $2 (21).
Spruce and fir, including hemlock, etc.	$0.75 (6, 7), $0.80 (8), $1 (5), $1.20 (9), $1.25 (4), $1.50 (2, 3, 13, 15, 16, 17), $1.60 (14), $1.75 (12), $2 (10), $2.25 (18, 19, 20). Eastern, $2 (21).
White pine	Western, $3.25 (2, 3), Eastern, $5 (21).
Subalpine	$0.50 (1, 2, 3, 4, 5, 6, 7, 8, 9, 16, 17), $1 (10, 12), $1.50 (15), $1.60 (14), $2.25 (18, 19, 20).
Hardwood	$1 (17), $1.50 (21).
Woodland	$0.50 (6, 7) (equivalent to $0.25 per cord). $1 (13, 14, 15, 16, 18, 19, 20).
Open	$0.50 (1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 13), $0.60 (12), $1 (14, 15, 16, 17, 18, 19, 20).

[a] Regions in parentheses.

[b] These prices are per cord.

proper methods have been worked out. However, costs are about the only tangible basis we have for valuing young stands.

In view of the many intangible values which can not be expressed in money, it seems fairly reasonable to use cost value for young growth as representing not only its value as potential timber but also the other forest values. This is on the theory that if the forest cover is to be maintained on a given site, it is worth at least what it would cost to put it there—if not for its timber value, then for other purposes, such as protection of watersheds. It seems quite possible that a portion of our Rocky Mountain forests will never yield enough timber to repay the costs of establishment and administration, unless timber values rise much higher than it seems reasonable to suppose. But because of their other values, which are of even greater importance, they will always be protected and maintained as forest. Cost of establishment as used here is not taken to mean the cost of growing the stand to maturity, or even to the age of that destroyed, but is merely the cost of getting young growth established. For use in figuring past losses, general values for the different types and regions were worked out by the following arbitrary method.[3]

Costs of replanting were set, based in part on results of planting operations on the national forests during a number of pre-war years, but mostly on arbitrary estimates of what replanting should cost if done immediately after a burn, and assuming that the proper technique had been developed (Table IX).

It was assumed that, taken by and large, one-half of the reproduction areas destroyed by fire will restock naturally within an average period of 10 years, and one-half not at all. Exceptions are lodgepole pine, jack pine, and the woodland types, of which it was assumed three-fourths will restock within 10 years and the rest not at all. Other exceptions are the western yellow pine type in the Great Basin and in the Southwest, and the scattered timber in the brush and grass types of all regions, of which it was assumed that but one-fourth will restock within 10 years.

The cost of restocking was then taken to be the cost of planting, plus compound interest at 3 per cent for 10 years. In case of destruction of young growth which will not restock naturally, the loss will be this figure; where natural restocking will take place the loss will be merely the 10 years' interest. This "rule-of-thumb" method gives the following results, "A" being area in acres and "C" the cost of planting per acre:

Where the entire area will restock naturally ..... 0.3439 AC, or 0.35 AC.
Where three-fourths of the area will restock naturally ..... .5939 AC, or 0.60 AC.
Where one-half of the area will restock naturally ..... .8439 AC, or 0.85 AC.
Where one-fourth of the area will restock naturally ..... 1.0939 AC, or 1.10 AC.

It is admitted that this method is not entirely scientific, but it is expected to give about as good a basis for valuing relative damage which has occurred over considerable areas and periods as we can get with the data available at present.

With the data described above (Tables VII, VIII, IX), the average monetary damage per acre was computed for the different types of forest and other cover within the several regions. (Table X.)

TABLE IX.—*Assumed costs of replanting, used as basis for estimating damage to young growth*

Forest type	Cost per acre to restock [a]
Western yellow pine, including sugar pine mixtures, etc.	$6 (3), $7 (2), $8.50 (11), $10 (1, 4, 6, 7, 8, 9, 10), $12 (12, 13, 16), $15 (14, 15, 17, 18, 19, 20).
Douglas fir, including western larch.	$6 (2), $7.50 (5, 6), $8 (3), $10 (1, 4, 7, 8, 9, 10, 12, 13, 16), $12 (18, 19, 20), $15 (14, 15), $20 (17).
White pine (W. and E.)	$5 (2), $6 (3), $6 (21). Norway pine, $6 (21).
Lodgepole pine	$8 (2, 3, 6), $10 (1, 4, 7, 8, 9, 10, 12, 13, 14, 15).
Jack pine	$10 (21).
Spruce	$5.50 (3), $6 (2), $6.50 (1, 9, 10), $8 (4, 7, 8), $10 (12, 13, 14, 15, 18, 19, 20).
Firs	$6 (2, 3, 21), $7.50 (4, 5, 6, 7, 8), $8 (16).
Subalpine	$6 (2, 3), $8 (4, 5, 6, 7, 8, 16), $10 (1, 9, 10, 12, 13, 14, 15).
Woodland	$5 (6, 12, 13, 14, 15, 16, 18, 19, 20).

[a] Regions in parentheses.

[3] A method for valuing young growth for use in making fire plans and in fire reports in the future is outlined in the discussion (pp. 759–760) on "Destructible values."

TABLE X.—*Average value of timber and young growth destroyed per acre burned over*

Region	Forest type								
	Western yellow pine and sugar pine	Douglas fir and larch	Lodge-pole pine	Spruce and firs	White pine	Sub-alpine	Wood-land	Brush	Grass
	Dollars	*Dollars*	*Dollars*	*Dollars*	*Dollars*	*Dollars*	*Dollars*	*Dollars*	*Dollars*
1		5.92	4.95			10.40		[a] 0.29	0.29
2	4.75	4.45	4.63	14.97	14.20	2.24		.29	.29
3	4.36	8.03	4.79	14.65	14.20	2.24		.29	.29
4	5.13	9.44	5.54	13.97		2.80		.47	.47
5		6.61		8.12		3.86			
6	6.15	4.82	2.97	4.72		1.40	1.22	.78	.04
7	5.27		3.18	3.76		1.40	.33	.78	.04
8	8.23	5.12	3.10	3.12		5.28		.94	.47
9		6.12	4.77	2.90		10.40		[a] .30	[a] .30
10		6.31	5.24	4.25		12.39		[a] .30	[a] .30
11	2.95							.65	.65
12	3.37	3.10	7.98	6.29		3.01			.28
13		3.20	8.50	5.97			.28	.28	.15
14	4.20	4.68	12.10	6.10			1.68	.53	.53
15	8.22	8.83	5.24	5.97		5.97	.48	.68	1.18
16	4.59		2.68	4.64		1.40	.82	.99	.05
17	7.12	6.75				1.40	[b] .09	.51	.51
18	4.74	3.62		4.71		4.71	1.68	.34	.40
19	4.74	3.62	8.50	4.71		4.71	1.68	.34	.40
20	9.18	3.62		4.71		4.71	1.68	.34	.40
21			[c] 1.85	[d] 1.20	1.20		[e] 1.20	.10	[a] .10

[a] Estimated, data unsatisfactory.
[b] Hardwood bottoms.
[c] Jack pine.
[d] Spruce, balsam, tamarack, value estimated same as eastern white pine.
[e] Hardwoods, value estimated same as eastern white pine.

RATING THE LIABILITY

Rating of the liability of a given individual unit involves two different processes. One is the rating of the general liability, or the liability due to general risk fires, which may be considered as an average figure generally applicable to the entire area of a given type within one region. The other process is the rating of the special liability due to special risk fires, which can not be applied generally but will be different for each specific unit. In each of these cases the rating should include the total liability of each sort, i. e., the liability of loss, plus the suppression liability.

GENERAL LIABILITY—Since the general risk has been assumed to be spread fairly evenly over the whole extension of a given type within one region, the general liability will be uniform for equal areas of the type, provided they are subject to the same "hour control," no matter which individual forest unit within that region may be under consideration. This liability will be the product of the sum of probable average loss plus probable average suppression cost per fire for the given hour control, multiplied by the average number of fires per year per unit of area of the given type, and can be computed as follows:

1. Average sizes of fires for different hour-control periods are shown in Figures 4–18.

2. Average damage per acre burned over is found inTable X. The products of these two sets of figures give average damage per fire.

3. Average suppression costs per fire for different hour control periods are given in Table VI.

4. The average numbers of general risk fires per 1,000 acres of each type and region are given in Table XI.

5. The sums of damages and costs, found as outlined above, multiplied by number of fires, gives the general liability per 1,000 acres for each type and region according to different hour controls. These values are given in Table XII.[4]

[4] Tables were also prepared for regions 1, 5, 7, 9, 10, 11, 13, 14, 15, 18, and 20, but are omitted because of the rather inadequate data on which they are based.

TABLE XI.—*Average number of general risk fires on national forests per year per 1,000 acres, by regions and forest types, 1911–1915*

Region	Forest types								
	Yellow pine, etc.	Douglas fir, etc.	Lodge-pole pine	Fir, spruce, etc.	White pine	Sub-alpine	Wood-land	Brush	Grass and sage
1		0.00582	0.00541			0.00418		0.00522	0.00522
2	0.03146	.01015	.00766	0.00952		.00569		.01231	.01231
3	.04059	.02391	.01843	.03113	0.06294	.04644		.01989	.01989
4	.05188	.02441	.01956	.04806		.01859		.10937	.10937
5		.01077	.01378	.04203		.01378			
6	.07316	.10559		.11850		.04215	0.02857	.09584	.13636
7	.06667		.01774	.08955		.04706	.01048	.02179	.01405
8	.03645	.01548	.01359	.00451		.00993		.00601	.01333
9		.00630	.00813	.02326		.00327		.00907	.00907
10		.00420	.00456	.00347		.00071		.00462	.00462
11	.05227							.01724	.01724
12	.01281	.03077	.00682	.00239		.00252	.01149	.01149	.01149
13		.03333	.00926	.00385			.00077	.00077	.00627
14	.00485	.00306	.00162	.00393		.00393	.00061	.00402	.00402
15	.02186	.02326	.00980	.00256		.00256	.00102	.00221	.00495
16	.05064	.05064	.00601	.07692		.01119	.02817	.04112	.07752
17	.05952	.03604				.03846	[a].13194	.02531	[b].25641
18	[c].04252	.04252		.04252		.04252	.00767	.00133	.00133
19	[c].00717	.00717	.00435	.00717		.00717	.00421	.00200	.00200
20	[c].03806	.03806		.03806		.03806	.00374	.00361	.00361
21			[d].01842	.00475	.06250		[e].01081	.10000	.10000

[a] Hardwood bottoms.
[b] Doubtful.
[c] Same figure for all timber types, because no basis for separating them.
[d] Jack pine.
[e] Hardwood.

TABLE XII.—*Total general liability per 1,000 acres by control periods*

REGION 2—WESTERN MONTANA

Hour control	Forest types						
	Western yellow pine	Douglas fir and larch	Western white pine[a]	Lodgepole pine	Spruce and fir	Subalpine	Open
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	0.79	0.24	3.52	0.14	0.14	0.07	0.19
1	1.26	.44	6.10	.21	.17	.10	.27
2	2.33	.85	8.50	.31	.21	.12	.40
3	3.02	1.27	10.51	.40	.24	.14	.50
4	3.65	1.57	12.65	.43	.28	.15	.60
5	4.28	1.85	15.11	.46	.32	.16	.68
6	4.94	2.07	17.25	.59	.36	.17	.75
7	5.57	2.35	19.76	.91	.41	.18	.84
8	6.20	2.69	23.41	1.19	.47	.20	.92
9	7.05	3.04	25.55	1.40	.52	.24	.99
10	7.90	3.41	29.52	1.62	.59	.42	1.07
12	9.50	4.08	32.86	2.07	.75	.65	1.23
15	12.46	5.12	39.21	2.87	.92	.84	1.53
20	17.71	6.78	52.18	4.43	1.34	1.06	2.09

[a] Region 3 figures used for white pine type, because data for this region are insufficient.

TABLE XII.—*Total general liability per 1,000 acres by control periods*—Contd.

REGION 3—NORTHERN IDAHO

Hour control	Forest types						
	Western yellow pine	Douglas fir and larch	Western white pine	Lodgepole pine	Spruce, cedar, fir, hemlock	Subalpine	Grass and brush
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	0.97	1.39	3.52	0.42	0.43	0.56	0.30
1	1.54	2.54	6.10	.57	.56	.79	.44
2	2.87	4.76	8.50	.81	.68	1.02	.66
3	3.68	5.92	10.51	1.08	.81	1.11	.82
4	4.41	7.65	12.65	1.12	.90	1.21	.98
5	5.22	8.70	15.11	1.22	1.06	1.30	1.09
6	5.99	9.45	17.25	1.53	1.15	1.39	1.21
7	6.75	10.28	19.76	2.40	1.31	1.49	1.35
8	7.52	11.41	23.41	3.07	1.46	1.62	1.49
9	8.57	12.22	25.55	3.54	1.68	2.00	1.59
10	9.68	13.22	29.52	4.09	1.93	3.45	1.73
12	11.65	15.23	32.86	5.29	2.43	5.34	1.99
15	15.08	18.53	39.21	7.17	2.99	6.87	2.47
20	21.43	24.91	52.18	11.00	4.36	8.68	3.38

TABLE XII.—*Total general liability per 1,000 acres by control periods*—Contd.

REGION 4—EASTERN WASHINGTON

Hour control	Forest types					
	Western yellow pine	Douglas fir and larch	Lodgepole pine	Spruce fir, hemlock, cedar	Subalpine	Grass and brush
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	1.56	0.81	0.59	1.54	0.22	5.91
1	2.39	1.17	.64	2.40	.32	7.77
2	3.71	1.66	.72	3.32	.45	10.06
3	6.48	2.27	1.00	3.92	.56	13.12
4	9.44	3.15	1.64	4.62	.59	15.97
5	12.92	5.10	2.05	5.29	.61	19.58
6	16.81	8.08	2.42	5.87	.63	23.30
7	21.37	13.86	2.82	6.44	.67	27.67
8	26.77	21.72	3.17	7.35	.69	32.33
9	32.32	32.03	3.66	8.27	.71	37.41
10	38.70	43.40	4.17	9.23	.74	42.33
12	52.24	72.45	5.36	10.19	1.04	54.70
15	78.28	129.96	7.12	12.69	1.73	72.08
20	130.05	264.53	11.11	16.77	3.27	116.05

REGION 6—SOUTHERN OREGON AND NORTHERN CALIFORNIA COAST RANGES

Hour control	Forest types						
	Western yellow pine	Douglas fir	Red and white fir	Subalpine	Digger pine and oak	Brush	Grass
	Dolls.	*Dolls*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	3.80	2.32	1.66	0.34	5.03	4.79	3.82
1	6.51	3.06	1.90	.34	6.71	6.80	4.77
2	10.83	4.65	2.25	.38	8.51	9.29	5.73
3	15.22	9.71	2.61	.38	10.11	12.08	6.70
4	19.68	14.36	2.84	.38	11.91	14.57	7.50
5	24.29	19.32	3.32	.42	13.89	16.96	8.45
6	28.90	23.76	5.10	.42	16.06	19.36	9.68
7	33.43	28.01	7.58	.42	18.46	21.56	10.77
8	38.04	31.57	8.89	.42	20.94	23.96	11.86
9	42.58	34.63	10.07	.46	23.49	26.36	13.09
10	47.04	38.36	10.90	.46	26.40	29.04	14.45
12	56.33	44.88	12.44	.51	32.54	35.17	17.45
15	64.45	56.38	14.70	.55	42.94	45.33	22.09
20	102.42	77.24	20.50	.76	63.77	64.70	31.23

TABLE XII.—*Total general liability per 1,000 acres by control periods*—Contd.

REGION 8—EASTERN OREGON AND SOUTHWESTERN IDAHO

Hour control	Forest types						
	Western yellow pine	Douglas fir and Larch	Lodgepole pine	Spruce, fir, etc.	Subalpine	Brush	Grass
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	1.45	0.28	0.30	0.12	0.13	0.60	0.72
1	2.22	.42	.34	.18	.19	.79	.93
2	4.05	.55	.37	.24	.27	1.05	1.23
3	6.49	.82	.50	.28	.34	1.37	1.60
4	9.59	1.14	.79	.32	.36	1.69	1.95
5	13.27	1.84	.95	.35	.38	2.07	2.37
6	17.50	2.90	1.13	.38	.40	2.48	2.84
7	22.38	4.97	1.29	.41	.42	2.95	3.37
8	28.17	7.89	1.44	.45	.44	3.45	3.96
9	34.51	11.36	1.64	.48	.45	3.99	4.56
10	41.08	15.32	1.86	.52	.48	4.53	5.16
12	55.92	25.28	2.35	.56	.78	5.83	6.65
15	84.38	44.82	3.10	.65	1.19	8.05	9.19
20	141.10	89.86	4.73	.79	2.33	12.45	14.13

REGION 12—EASTERN COLORADO

Hour control	Forest types					
	Western yellow pine	Douglas fir	Lodgepole pine	Engelmann spruce	Subalpine	Grass and brush
	Dolls	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	0.24	0.86	0.25	0.08	0.02	0.18
1	.31	1.11	.42	.10	.03	.26
2	.42	1.88	.83	.13	.05	.38
3	.50	2.52	1.21	.17	.07	.47
4	.55	3.08	1.71	.19	.08	.56
5	.67	3.72	2.31	.22	.09	.67
6	.78	4.46	2.90	.25	.11	.77
7	1.04	5.26	3.61	.28	.12	.89
8	1.39	6.09	4.36	.31	.13	1.00
9	1.79	6.99	5.22	.35	.14	1.15
10	2.21	8.00	6.05	.39	.15	1.29
12	3.20	10.06	8.13	.46	.16	1.60
15	5.05	13.91	11.63	.60	.21	2.16
20	9.49	21.35	19.19	.84	.28	3.25

TABLE XII.—*Total general liability per 1,000 acres by control periods*—Contd.

REGION 16—WEST SLOPE OF SIERRAS

Hour control	Forest types						
	Western yellow and sugar pines	Red and white fir	Lodgepole pine	Subalpine	Digger pine and oak	Brush	Grass
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	2.08	0.92	0.20	0.01	3.80	6.43	4.50
1	4.15	1.69	.38	.01	4.85	7.76	5.27
2	7.04	2.54	.62	.02	5.97	9.88	5.97
3	9.72	2.77	.83	.02	7.04	11.62	6.59
4	12.26	2.92	1.03	.02	8.28	13.16	7.29
5	14.79	3.08	1.23	.02	9.61	14.45	7.91
6	16.66	3.23	1.37	.02	11.04	15.90	8.60
7	18.28	3.38	1.50	.02	12.34	17.62	9.23
8	19.65	3.46	1.60	.02	14.25	19.37	10.00
9	21.32	3.54	1.73	.02	15.97	21.05	10.54
10	22.99	3.69	1.86	.03	17.72	22.98	11.18
12	25.57	3.92	2.04	.03	20.47	27.22	12.35
15	30.74	4.15	2.33	.04	27.92	33.56	14.19
20	41.07	4.77	3.21	.09	41.32	46.77	18.22

REGION 17—SOUTHERN CALIFORNIA

Hour control	Forest types					
	Western yellow pine	Fir and pine slopes	Subalpine	Hardwoods	Chaparral	Grass,[a] etc.
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls*	*Dolls.*
½	2.92	3.31	0.04	4.22	6.25	21.28
1	5.24	7.62	.04	7.26	7.79	28.97
2	10.54	17.44	.08	11.34	9.47	36.15
3	16.25	25.51	.08	14.65	11.13	44.62
4	21.49	33.33	.08	16.62	12.68	54.62
5	26.90	41.19	.08	17.94	14.35	60.51
6	32.80	51.28	.08	18.60	16.02	76.67
7	38.45	62.57	.08	19.26	17.90	88.97
8	43.75	75.10	.08	19.92	20.00	102.82
9	48.28	87.60	.08	20.58	22.15	117.18
10	53.10	101.36	.11	21.62	24.28	134.10
12	62.14	132.11	.11	23.22	29.03	167.95
15	79.70	186.34	.15	25.33	37.21	229.49
20	115.42	298.27	.31	28.63	53.00	349.49

[a] Data on area very unsatisfactory; figures doubtless too high.

TABLE XII.—*Total general liability per 1,000 acres by control periods*—Contd.

REGION 19—SOUTHWESTERN COLORADO AND NORTHERN NEW MEXICO

Hour control	Forest types						
	Yellow pine	Douglas fir	Spruce and subalpine	Lodgepole pine	Pinon-juniper	Brush	Grass
	Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
½	0.19	0.13	0.32	0.16	0.01	0.02	0.03
1	.31	.21	.48	.28	.01	.02	.05
2	.60	.38	.61	.55	.02	.03	.08
3	.97	.65	.92	.80	.02	.03	.12
4	1.41	.93	1.06	1.14	.02	.04	.17
5	1.93	1.27	1.21	1.54	.03	.05	.23
6	2.53	1.66	1.32	1.94	.03	.06	.30
7	3.20	2.09	1.46	2.41	.03	.06	.38
8	3.98	2.57	1.60	2.92	.03	.07	.47
9	4.85	3.10	1.73	3.51	.04	.08	.57
10	5.72	3.66	1.86	4.07	.05	.09	.68
12	7.99	4.88	2.17	5.47	.05	.12	.92
15	11.81	7.27	2.60	7.84	.06	.16	1.37
20	21.41	12.27	3.41	12.95	.09	.23	2.29

REGION 21—LAKE STATES[a]

Hour control	Forest types				
	Eastern white and red pines	Jack pine	Spruce, balsam, tamarack	Hardwood	Open
	Dollars	*Dollars*	*Dollars*	*Dollars*	*Dollars*
½	3.62	13.96	0.28	0.63	0.80
1	5.00	20.83	.38	.86	1.00
2	8.19	28.53	.62	1.42	1.20
3	12.12	37.97	.92	2.10	1.60
4	17.00	49.98	1.29	2.94	1.90
5	22.62	61.97	1.72	3.91	2.30
6	28.75	75.67	2.19	4.97	2.80
7	35.75	92.12	2.72	6.18	3.20
8	43.56	109.23	3.31	7.53	3.70
9	52.12	128.73	3.96	9.02	4.20
10	61.19	147.51	4.65	10.58	4.90
12	82.00	196.29	6.23	14.18	6.10
15	119.31	263.87	9.07	20.64	8.30
20	196.50	419.46	14.93	33.99	12.70

[a] All figures on areas very doubtful, and these values are therefore not very satisfactory.

To use these figures in estimating the general liability of a given forest unit it will be necessary to know the area and location of the different types of forest cover within the unit, and the hour-control that will be effected for all parts of the unit with the existing or proposed protective organization. Thus, the fire plan might show a forest in western Montana something like this:

Forest type	Area	Hour control	General liability
	Acres	*Hours*	*Dollars*
Western yellow pine	100,000	Less than 1	79.00
	50,000	2	116.50
	50,000	3	151.00
	50,000	4	182.50
Douglas fir	50,000	2	42.50
	100,000	4	157.00
	200,000	6	414.00
	50,000	12	204.00
Lodgepole pine	100,000	6	59.00
	50,000	8	59.50
Subalpine	50,000	10	21.00
	50,000	15	42.00
Total general liability for the forest			1,528.00

This means that, with the given amount of protection, the average annual loss plus suppression cost for general risk fires, for a period of years, ought to be about $1,500; some years might run above, others below this figure. It is hardly necessary to say that the example given is for a purely imaginary forest.

SPECIAL LIABILITY.—Rating of the special liability will be done in a slightly different way. It is not uniform for the whole area of a given type within a region, but is confined only to those parts of the type which are exposed to the fixed risks and varies for different units as the chance of occurrence of fires varies. In order to rate a unit it will be necessary to know what areas of each type are exposed to special risks, how many fires per year can be expected in each, and what hour control will be provided by the existing or proposed protection organization. The number of fires per year will be based on the average number which have occurred in that particular unit during a period of years, making due allowance for changes in the hazard, such as adoption of spark arresters or of fuel oil, or construction of effective fire lines along railroads. The special liability will then be the products of losses plus costs per fire for different hour control periods, found in the same way as described under general liability, multiplied by the number of special risk fires. For example, let us take the hypothetical western forest already described. We find that areas exposed to special risks are as follows:

Class of risk, forest type, and area	Hour control	Fires per year	Loss plus cost per fire	Product
Railroad fires:				
Yellow pine type—	*Hours*	*Number*	*Dollars*	*Dollars*
50,000 acres	Less than 1	25	25.25	631.25
20,000 acres	2	10	74.50	745.00
Douglas fir type—				
20,000 acres	2	5	83.60	418.00
Total liability due to railroad				1,794.25
Lumbering operations:				
Yellow pine type—				
25,000 acres	Less than 1	8	25.25	202.00
Douglas fir type—				
50,000 acres	2	7	83.60	585.20
Total liability due to lumbering				787.20
Total special liability				2,581.45

TOTAL GENERAL AND SPECIAL LIABILITY.—Having found the general and the special liabilities for the unit in the manner described, the total liability from all causes is their sum, or in the illustration given, $1,528 plus $2,581.45, or $4,109.45. It will be noticed that nowhere in this method of rating has any allowance been made for variations in the factor of efficiency. This appears to be justified for at least two reasons. The grouping together of the fires on several of the national forests and for periods of several years in studying the past records should have evened out differences in efficiency as far as past performance is concerned, and in figuring on future organizations we should assume these differences to grow no greater, and probably less.

LENGTH OF FIRE SEASON

The cost of maintaining a protective organization will be governed partly by the length of the period during which it must be effective. This of course depends upon the length of the danger period in the different units. The occurrence of general risk fires per unit of area (per 1,000,000 acres) in the different types and subregions, by 10-day periods, is shown in Table XIII. If it be assumed that the fire season, the period during which the protective organization should be effective, is marked by the period during which more than one fire per million acres occurs in each 10-day period, the fire seasons for the different types and regions will be found indicated under "Fire danger A" in each section of the table. A smaller number of fires per 10-day period ought to be handled effectively by the regular administrative organization, without seriously interfering with their other work. If the standard is set at some point greater than one per 10 days, it will cut down the fire seasons accordingly. (See "Fire danger B," Table XIII.)

TABLE XIII.—*Average number of general risk fires per year, per million acres, by 10-day periods* [a]

REGION 2—WESTERN MONTANA

Period	Number of fires, by forest types						
	Western yellow pine	Douglas fir and larch	Western white pine	Lodgepole pine	Spruce and fir	Subalpine	Grass and brush
Apr. 21–30				0.2			
May 1–10		0.1					
11–20	0.2						0.6
21–31		.1					
June 1–10	.2	.1					
11–20	.5	.2		0.1		0.2	
21–30	1.4	.8		.5		.2	.6
July 1–10	.2	.1		.2		.3	.6
11–20	2.5	.5		.3	0.6	.2	1.3
21–31	4.1	2.5		.7	2.5	.6	.6
Aug. 1–10	6.0	3.1	3.0	2.1	1.3	1.0	1.3
11–20	8.3	2.3		1.2		1.8	3.2
21–31	6.9	3.3		1.6	1.3	1.3	3.2
Sept. 1–10	1.6	.4		.8	1.3		.6
11–20	.9	.1		.1			.6
21–30	1.2	.1					.6
Oct. 1–10				.1			
11–20		.1					
21–31	.2						
Nov. 1–10							
11–20							
21–30		.1					
Danger periods A.[b]	June 21–30; July 11–Sept. 30.	July 21–Aug. 31.	Aug. 1–10.	Aug. 1–31.	July 21–Sept. 10.	Aug. 1–31.	July 11–20; Aug. 1–31.
Danger periods B.[b]	July 21–Aug. 31.	Aug. 1–31.	Aug. 1–10.	None.	None.	None.	Aug. 11–31.

[a] Tables were prepared also for Regions 1, 5, 7, 9, 10, 11, 13, 14, 15, 18, and 20, but are omitted because of the rather inadequate data on which they were based.

[b] Danger periods A are the periods during which one or more than one fire occurs in 10 days. Danger periods B are the periods within which three or more fires occur in 10 days, or two fires or more a week.

TABLE XIII.—*Average number of general risk fires per year, per million acres, by 10-day periods*—Continued

REGION 3—NORTHERN IDAHO

Period	Number of fires, by forest types						
	Western yellow pine	Douglas fir and larch	Western white pine	Lodgepole pine	Spruce, hemlock, fir, cedar	Subalpine	Brush and grass
Apr. 11–20	0.9						
21–30							
May 1–10					0.8		
11–20		0.1	0.2				
21–31	.9						
June 1–10		.1	.4				
11–20		.3	.6				0.4
21–30	.9	.1	1.2		1.6		
July 1–10	.9	.2		0.9		0.6	
11–20		1.0	4.2	.9	1.6	1.7	.4
21–31	9.8	5.7	8.6	2.8	2.4	4.4	2.6
Aug. 1–10	6.2	3.2	13.0	6.6	7.2	3.8	1.8
11–20	8.0	4.4	13.2	5.6	6.4	12.9	5.7
21–31	16.9	5.3	17.0	2.8	4.0	10.2	4.8
Sept 1–10	8.0	1.7	4.2		4.8	2.6	1.3
11–20	1.8	.1	.6			.3	
21–30							.4
Danger periods A [b]	July 21–Sept. 20	July 11–Sept. 10	June 21–30; July 11–Sept. 10	July 21–Aug. 31	June 21–30; July 11–Sept. 10	July 11–Sept. 10	July 21–Sept. 10
Danger periods B [b]	July 21–Sept. 10	July 21–Aug. 31	July 11–Sept. 10	Aug. 1–20	Aug 1–Sept. 10	July 21–Aug. 31	Aug. 11–31

REGION 4—EASTERN WASHINGTON

Period	Number of fires, by forest types					
	Western yellow pine	Douglas fir and larch	Lodgepole pine	Spruce, hemlock, cedar, white fir	Subalpine	Grass and brush [c]
May 11–20	0.6					
21–31						
June 1–10	1.7	0.5				
11–20	2.8	.2		1.6		2.0
21–30	7.8	1.2	4.2	1.6	0.5	2.0
July 1–10	7.8	2.2	4.2	3.1	2.3	
11–20	6.2	2.7		2.3	.9	
21–31	17.4	4.9		8.6	9.2	2.0
Aug. 1–10	7.3	4.4	4.2	7.8	2.3	
11–20	6.2	4.4		11.7	1.4	2.0
21–31	11.8	5.9	12.6.	18.7	.9	6.0
Sept. 1–10	6.7	1.9		3.9		6.0
11–20	3.9			1.6		
21–30	2.2			.8	.5	6.0
Oct. 1–10	2.8	.2				
11–20						2.0
21–31	.6					
Nov. 1–10	.6					
Danger periods A [b]	June 1–Oct. 10	June 21–Sept. 10	June 21–Aug 31	June 11–Sept. 20	July 1–Aug. 20	June 11–Oct. 20
Danger periods B [b]	June 21–Sept. 20	July 21–Aug. 31	Same as A.	July 1–Sept. 10	July 21–31	Aug. 21–Sept. 30

[b] Danger periods A are the periods during which one or more than one fire occurs in 10 days. Danger periods B are the periods within which three or more fires occur in 10 days, or two fires or more a week.
[c] Data on area of this type very unsatisfactory, therefore figures are little better than a guess.

TABLE XIII.—*Average number of general risk fires per year, per million acres, by 10-day periods*—Continued

REGION 6—SOUTHERN OREGON AND NORTHERN CALIFORNIA COAST RANGES

Period	Number of fires, by forest types							
	Western yellow pine	Western yellow and sugar pines	Douglas fir	Fir and lodge-pole	Sub-alpine	Digger pine and oak	Brush	Grass
Mar. 11–20		0.2						
21–31								
Apr. 1–10		.3						
11–20		.3					0.2	
21–30		.5						
May 1–10		.5	0.3				.2	
11–20	1.8	1.2	.8	0.7			.4	
21–31	1.0	1.9	.3	2.7		0.9	.6	
June 1–10	2.8	2.6	1.1	.7		.9	1.1	4.4
11–20	1.0	1.6		1.3			.4	
21–30		1.0	.3	.7			.6	
July 1–10	5.3	7.7	3.1	8.0		.9	4.4	13.2
11–20	3.9	6.1	6.2	4.0	3.9	2.8	3.2	13.2
21–31	7.4	9.5	7.8	7.4	3.9	.9	5.6	4.4
Aug. 1–10	6.8	4.7	4.8	3.3		11.4	8.9	8.8
11–20	4.2	9.1	8.1	10.7	3.9	.9	11.6	13.2
21–31	7.7	10.2	20.7	22.8	11.7	9.5	13.8	13.2
Sept. 1–10	1.8	4.6	5.9	1.3		2.8	4.4	4.4
11–20	1.8	2.1	2.2	2.0		1.9	8.4	
21–30	1.8	3.3	3.9	1.3		4.7	4.7	
Oct. 1–10	.4	3.3	2.8	4.0			6.8	
11–20	1.4	2.3	7.0	2.0			7.0	4.4
21–31	1.0	1.4	.6			.9	1.7	30.8
Nov. 1–10	.4	.2					.6	
11–20	.4	.7						
21–30	1.4	.2					.8	
Danger periods A [b]	May 11–June 20; July 1–Sept 30; Oct. 11–31; Nov. 21–30	May 11–Oct. 31	June 1–10; July 1–Oct. 20	May 21–31; June 11–20; July 1–Oct. 20	July 11–31; Aug. 11–31	July 11–Sept 30	June 1–10; July 1–Oct. 31	June 1–10; July 1–Sept. 10; Oct. 11–31
Danger periods B [b]	July 1–Aug. 31	July 1–Sept. 10; Sept. 21–Oct. 10	July 1–Sept. 10; Sept. 21–30; Oct. 11–20	July 1–Aug. 31; Oct. 1–10	Same as A	Aug. 1–10; Aug. 21–31; Sept 21–30	July 1–Oct. 20	Same as A

REGION 8—EASTERN OREGON AND SOUTHWESTERN IDAHO

Period	Number of fires, by forest types						
	Western yellow pine	Douglas fir and larch	Lodge-pole pine	Spruce, white fir (cedar)	Subalpine	Brush	Grass and sage
Apr. 21–30	0.1						
May 1–10	.1						
11–20							
21–31	.3						
June 1–10							
11–20	.5						
21–30	.5		0.1				0.3
July 1–10	.5	0.1	.2		0.3		.7
11–20	1.2	.2	.6			1.0	1.3
21–31	3.6	1.5	1.9	0.5	.6		.7
Aug. 1–10	7.4	3.1	1.9	2.5	1.1	1.0	1.7
11–20	7.7	2.4	2.4	.5	2.2	2.0	1.7
21–31	6.9	2.9	1.9		1.9	1.0	3.0
Sept. 1–10	4.0	1.8	2.0		.8		2.3
11–20	.9	.9	.2	.5	.6		.7
21–30	.5	.3	.1		.5		
Oct. 1–10	.3		.3				
11–20	.1						
21–31	.1	.1	.1				
Nov. 1–10	.1						
Danger periods A [b]	July 11–Sept. 10	July 21–Sept. 10	July 21–Sept. 10	Aug. 1–10	Aug. 1–31	July 11–Aug. 31	July 11–Sept. 10
Danger periods B [b]	July 21–Sept 10	Aug. 1–10	None.	None.	None.	None.	Aug. 21–31

[b] Danger periods A are the periods during which one or more than one fire occurs in 10 days. Danger periods B are the periods within which three or more fires occur in 10 days, or two fires or more a week.

TABLE XIII.—*Average number of general risk fires per year, per million acres, by 10-day periods*—Continued

REGION 12—EASTERN COLORADO

Period	Number of fires, by forest types					
	Western yellow pine	Douglas fir	Lodgepole pine	Engelmann spruce	Subalpine	Grass and brush
Mar. 21–31						0.9
Apr. 1–10	0.2					
11–20				0.2		
21–30						
May 1–10	.2		0.4			
11–20						
21–31	1.2	3.1		.5		
June 1–10	.7		.4		4.0	
11–20	.5	3.1	.4	.2	4.0	.9
21–30	.9		1.3	.2	4.0	2.6
July 1–10	2.3	9.2	1.8	.2	8.0	.9
11–20	.9		.9			
21–31	.7	3.1	.4	.5		
Aug. 1–10	1.6	3.1	.9			.9
11–20	.7					.9
21–31	.7	6.1	.4		4.0	
Sept. 1–10	.7	3.1	1.3			
11–20	.5		.4			.9
21–30	.2		.4			
Oct. 1–10	.9	3.1				.9
11–20	.5	3.1				
21–31			.4	.2		
Nov. 1–10				.2		.9
11–20	.7		.4	.2		
21–30						.9
Danger periods A [b]	May 21–31; July 1–10; Aug. 1–10.	May 21–31; June 11–20; July 1–10; July 21–Aug. 10; Aug. 21–Sept. 10; Oct. 1–20.	June 21–July 10; Sept. 1–10.	None.	June 1–July 10; Aug 21–31.	June 21–30.
Danger periods B [b]	None.	Same as A.	None.	None.	Same as A.	None.

[b] Danger periods A are the periods during which one or more than one fire occurs in 10 days. Danger periods B are the periods within which three or more fires occur in 10 days, or two fires or more a week.

TABLE XIII.—*Average number of general risk fires per year, per million acres, by 10-day periods*—Continued

REGION 16—WEST SLOPE OF SIERRAS

Period	Number of fires, by forest types						
	Western yellow pine	Western yellow and sugar pines and Douglas fir	Red and white fir	Lodgepole pine and subalpine	Digger pine and oak	Brush	Grass
Feb. 21–28/29		0.5				0.2	
Mar. 1–10	0.3						
11–20	1.0	.1					
21–31							
Apr. 1–10							
11–20	.3						
21–30		.2					
May 1–10		.1					
11–20	.8	.2					1.7
21–31	1.3	.4			1.0	0.2	1.7
June 1–10	3.1	2.0	1.5		3.1	1.3	
11–20	4.2	1.3	3.0	0.3	3.1	.4	3.4
21–30	1.0	1.2		.6		.6	1.7
July 1–10	3.9	1.3	4.0			1.5	13.6
11–20	4.9	4.4	12.0	.3		4.4	8.5
21–31	7.0	4.5	7.0	.6	1.0	4.1	8.5
Aug. 1–10	9.4	3.9	8.5	.8	5.2	5.7	10.2
11–20	10.4	4.5	7.0	1.1	2.1	5.0	10.2
21–31	14.8	9.8	16.5	2.2	4.2	8.3	5.1
Sept. 1–10	5.2	4.1	6.5	.6	2.1	5.2	3.4
11–20	7.3	4.9	7.0	.6	3.1	5.2	1.7
21–30	12.0	3.3	6.5	.6	1.0	3.5	5.1
Oct. 1–10	4.7	1.7	3.0	.3		4.1	3.4
11–20	4.9	1.3	1.0		1.0	2.6	5.1
21–31	2.9	2.6			1.0	.9	
Nov 1–10	1.0	.6	.5				
11–20	.3					.2	1.7
21–30	.3	.2				.2	1.7
Dec. 1–10		.2					
11–20		.1					
Danger periods A.[b]	May 21–Nov. 10.	June 1–Oct. 31.	June 1–20; July 1–Oct. 20.	Aug. 11–31.	May 21–June 20; July 21–Sept. 30; Oct. 11–31.	June 1–10; July 1–Oct. 20.	May 11–31; June 11–Oct. 20; Nov. 11–31.
Danger periods B.[b]	June 1–20; July 1–Oct. 20.	July 11–Sept. 30.	June 11–20; July 1–Oct. 10.	None.	June 1–20; Aug. 1–10; 21–31; Sept. 11–20.	July 11–Oct. 10.	June 11–20; July 1–Sept. 10; Sept. 21–Oct. 20.

[b] Danger periods A are the periods during which one or more than one fire occurs in 10 days. Danger periods B are the periods within which three or more fires occur in 10 days, or two fires or more a week.

TABLE XIII.—*Average number of general risk fires per year, per million acres, by 10-day periods*—Continued

REGION 17—SOUTHERN CALIFORNIA

Periods	Number of fires, by forest types				
	Western yellow and Jeffrey pines	Fir and pine slopes	Hardwood bottoms	Chaparral	Grass and sage [d]
Jan. 1-10	0.6			0.2	
11-20					5.0
21-31				.1	
Feb. 1-10				.1	
11-20				.1	
21-28/29				.3	
Mar. 1-10					
11-20				.1	
21-31			1.4	.1	5.0
Apr. 1-10				.1	
11-20	0.6			.1	
21-30				.2	
May 1-10		1.8		.3	5.0
11-20	.6		2.8	.1	
21-31	2.4		1.4	.2	10.0
June 1-10	1.2		2.8	.2	5.0
11-20	1.8		7.0	.9	45.0
21-30	2.4	5.4	12.6	.8	25.0
July 1-10	1.8	1.8	14.0	1.5	20.0
11-20	5.4	1.8	5.6	1.5	15.0
21-31	6.0	3.6	2.8	3.2	50.0
Aug. 1-10	10.8	5.4	9.8	2.6	5.0
11-20	6.0	5.4	23.8	2.7	15.0
21-31	9.0	18.0	21.0	2.8	20.0
Sept. 1-10	4.8	12.6	22.4	1.7	25.0
11-20	7.8	7.2	5.6	2.0	20.0
21-30	1.8	1.8	1.4	2.0	5.0
Oct. 1-10	1.8		1.4	1.0	15.0
11-20	.6		2.8	2.1	15.0
21-31	1.8	1.8	4.2	1.0	25.0
Nov. 1-10	1.2		2.8	.8	20.0
11-20	1.2	3.6	2.8	.7	10.0
21-30	1.2		1.4	1.3	5.0
Dec. 1-10				.4	
11-20				.1	
21-31				.1	5.0
Danger periods A [b]	May 21-Oct. 10; Oct. 21-Nov. 30.	May 1-10; June 21-Sept. 30; Oct. 21-31; Nov. 11-20.	Mar. 21-31; May 11-Nov. 30.	July 1-Oct. 31; Nov. 21-30	Jan. 11-20; Mar. 21-31; May 1-Nov. 30; Dec. 21-31.
Danger periods B [b]	July 11-Sept. 20.	June 21-30; July 21-Sept. 20; Nov. 11-20.	June 11-Sept. 20; Oct. 21-31.	July 21-31.	Same as A

[b] Danger periods A are the periods during which one or more than one fire occurs in 10 days. Danger periods B are the periods within which three or more fires occur in 10 days, or two fires or more a week.
[d] Data on area very unsatisfactory, figures probably too high.

TABLE XIII.—*Average number of general risk fires per year, per million acres, by 10-day periods*—Continued

REGION 19—SOUTHWESTERN COLORADO AND NORTHERN NEW MEXICO [e]

Period	Number of fires, by forest types			
	Western yellow pine, Douglas fir, Engelmann spruce	Lodgepole pine	Pinon-juniper	Grass, brush, sage, and aspen
Mar. 1–10			0.4	
11–20				
21–31				
Apr. 1–10				
11–20				
21–30	0.2			
May 1–10	.2		.4	
11–20	.3		.7	0.3
21–31	.7			
June 1–10	.8		.7	.1
11–20	.4			.1
21–30	.8			.2
July 1–10	.6	0.9	.7	.1
11–20	.6			
21–31	.1			.1
Aug. 1–10	.2			.1
11–20	.5			.1
21–31	.5		.4	
Sept. 1–10	.1			
11–20	.1			
21–30	.2			.1
Oct. 1–10				.1
11–20	.2			
21–31	.4			.4
Nov. 1–10				
11–20	.1			.1
21–30	.3			.1

REGION 21—LAKE STATES [f]

Period	Number of fires, by forest types				
	Eastern white and red pines	Jack pine	Hardwoods	Tamarack and balsam	Open
Apr. 1–10	1.2				
11–20	2.5	1.5			6.7
21–30	13.7	3.7			13.3
May 1–10	3.7	.7			20.0
11–20	8.7	3.0	1.1		6.7
21–31	2.5	2.2	1.1		
June 1–10	5.0	1.1	4.3		
11–20	2.5	1.1	2.2		
21–30	1.2	1.5	1.1		6.7
July 1–10	1.2	.7	1.1		6.7
11–20	5.0	1.8	1.1		6.7
21–31	1.2				
Aug. 1–10		1.8			
11–20		.7		1.0	
21–31	1.2				6.7
Sept. 1–10	1.2	.4			6.7
11–20				1.0	
21–30					
Oct. 1–10		.7			6.7
11–20					
21–31		.4			
Danger periods A [b]	Apr. 1–July 31; Aug. 21–Sept. 10.	Apr. 11–30; May 11–June 30; July 11–20; Aug. 1–10.	May 11–July 20.	Aug. 11–20; Sept. 11–20.	Apr. 11–May 20; June 21–July 20; Aug. 21–Sept. 10; Oct. 1–10.
Danger periods B [b]	Apr. 21–May 20; June 1–10; July 11–20.	Apr. 21–30; May 11–20.	June 1–10.	None.	Same as A.

[b] Danger periods A are the periods during which one or more than one fire occurs in 10 days. Danger periods B are the periods within which three or more fires occur in 10 days, or two fires or more a week.

[e] No danger periods occurred in this region; that is to say, the average per 10 days was always less than one fire.

[f] Data on areas of all types very unsatisfactory; figures for numbers of fires in "open" probably much too high.

THE USE OF LIABILITY RATINGS IN FIRE PLANS

It is realized that the figures given in Tables VI to XIII are based on such incomplete data in many cases, perhaps in all, that they can not be used as absolute guides in allotting funds for primary protection. It does seem reasonable to believe, however, that figures worked out in this way can be so used, as soon as sufficient data accumulate to afford a basis for reliable figures on spread of fires, on suppression costs, and on the damage done in different types. It will also be desirable, perhaps, to have a more detailed classification of fires based not only on mere segregation by types, but also according to differences in the age of the stands, differences in quality of sites, and differences in characteristics with respect to inflammability.

Meanwhile, the figures given here may serve as valuable indicators in planning protection, provided they are not relied upon to too great an extent. In the first place, as fire plans for each of the national forests are worked out, showing the locations and areas of the different forest types classified according to the hour control now in effect, and ratings are made by the use of the tables, great differences in liabilities between different forests will undoubtedly appear. It will then be proper to examine more closely those forests whose liability is rated especially high and extremely low, to see whether or not more protection should be given the former.

Then, if the ratings could be relied upon absolutely, the justification of a suggested increase or decrease in protection could be determined by weighing its cost against the reduction or increase in total liability effected by such modification of the protection organization. Ratings based on the present data are not good enough to decide such questions, but should at any rate be suggestive.

A point which should be borne in mind is that it may not always be necessary to increase expenditures in order to increase the intensity of protection or to reduce the hour control. This may be accomplished on any forest unit in other ways, such as changing the distribution of personnel so as to locate men nearest to where the greatest number of fires will start, or nearest to where fires may be expected to spread most rapidly or be most destructive or costly to control, such as slashings, for instance. Nor do increased protection expenditures necessarily mean increased personnel, but the expenditure may be made in such a way as to reduce the hazard, by isolating special risks, or by removing especially hazardous conditions, such as logging slash, windfalls, or snags, or by improving communication.

MINIMUM REQUIREMENTS

It is believed that one exception should be made to the general principle of weighing costs against liabilities; that is, except in a very few places where it is certain that fires can be left without danger, enough protection should be provided during the danger season so that it will be possible to reach any part of every forest unit within 12 hours after a fire is discovered. The reason for this is that the law of averages is less dependable for longer elapsed periods, and even though averages may show comparatively low liabilities, it is more than likely that a considerable proportion of fires left for longer periods may do a great deal of damage or may prove very costly to control, or that they will spread from areas of low liability to areas where damage and costs will be much greater. A large proportion of the worst fires that have occurred on the national forests burned for more than 12 hours before they were attacked, and a considerable part of the total fire loss has been caused by such fires. For instance, nearly half of the total timber area burned during the five years studied, when the protective organization was not as well developed as it has subsequently become, is shown by the available records of elapsed time to have been burned over by fires which were not attacked until 12 hours or more after their discovery. (Tables XIV, XV, and XVI.)

TABLE XIV.—*Comparison of areas on which fires were attacked within 12 hours of discovery and those on which attack was later*

Region	Acreage of timber fires [a] by time of attack				Acreage of woodland and open fires by time of attack			
	Attack within 12 hours	Attack after 12 hours	Total burned	Percentage after 12 hours	Attack within 12 hours	Attack after 12 hours	Total burned	Per centage after 12 hours
	Acres	*Acres*	*Acres*	*Per cent*	*Acres*	*Acres*	*Acres*	*Per cent*
1	3,975	94	4,069	2	788	10	798	1
2	13,316	7,659	20,975	37	600	0	600	0
3	8,837	5,386	14,223	38	73	83	156	53
4	8,871	22,655	31,526	72	4,160	4,940	9,100	54
5	8,272	15,724	23,996	66				
6	17,477	7,154	24,631	29	22,274	7,573	29,847	25
7	6,940	12,168	19,108	64	11,295	0	11,295	0
8	8,961	27,264	36,225	75	2,948	4,600	7,548	61
9	5,901	813	6,714	12	3,894	161	4,055	4
10	6,083	1,471	7,554	19	12,438	7,968	20,406	39
11	26,657	827	27,484	3	2,506	200	2,706	7
12	1,484	0	1,484	0	96	20	116	17
13	2,602	451	3,053	15	1,566	361	1,927	19
14	21	80	101	79	1,070	1,282	2,352	54
15	487	153	640	24	12,706	60	12,766	0
16	29,084	4,565	33,649	14	67,567	10,281	77,848	13
17	6,228	590	6,818	9	147,539	6,617	154,156	4
18	17,294	32,090	49,384	65	8,578	87	8,665	1
19	3,058	648	3,706	17	2,287	0	2,287	0
20	8,514	670	9,184	7	8,893	555	9,448	6
21	[b]	[b]	[b]	[b]	66,593	15,149	81,742	19
Total	184,062	140,462	324,524	43	377,871	59,947	437,818	14

[a] Including subalpine type.

[b] Included in open.

TABLE XV.—*Number of fires by elapsed time groups, for general risk fires in all types*

Region	Total number of fires	Fires by hours elapsed from discovery to start of suppression work												
		Less than 1	1 to 2	2 to 3	3 to 4	4 to 5	5 to 6	6 to 7	7 to 8	8 to 9	9 to 10	10 to 15	15 to 20	More than 20
1	42	15	9	2	2	2	1		1	2	2	2	2	2
2	337	104	58	17	17	24	14	6	12	13	10	16	19	27
3	588	174	62	47	17	19	11	9	19	23	12	68	44	83
4	330	197	29	15	8	4	5	4	7	4	7	18	13	19
5	83	31	4	6	2		4	2	1	4	1	8	8	12
6	1,499	447	251	183	112	75	51	58	38	44	24	106	56	54
7	828	285	129	75	41	50	33	20	16	15	23	61	47	33
8	465	130	57	45	36	20	17	14	18	6	19	35	31	37
9	116	51	19	8	7	4	4		3	3	3	3	7	4
10	131	51	16	7	16	6	6	4	3	1	1	8	3	9
11	434	211	90	47	21	15	9	10	8	2	2	12	6	1
12	108	71	9	6	6	3	3	2	1	2	1	2	2	
13	108	56	19	6	8	3	1	2	4	1	1	2	1	4
14	25	11	6	2	2			1				1		2
15	73	27	12	12	3	4	2	1	3	1	4	1	1	2
16	1,385	669	223	135	79	52	20	25	15	17	20	77	27	26
17	745	557	78	22	21	18	6	9	6	5	4	10	3	6
18	1,096	496	154	129	85	52	27	23	17	10	11	38	30	24
19	235	114	40	22	18	10	5	3	4	1	3	6	5	4
20	197	76	43	17	16	8	9	4	1	4	1	7	4	7
21	149	105	13	8	9	1			1	1		5	3	3
Total	[a]8,974	3,878	1,321	811	526	370	228	197	178	159	149	486	312	359
Per cent	100	43.2	14.7	9.0	5.9	4.1	2.5	2.2	2.0	1.8	1.7	5.4	3.5	4.0

[a] Totals of Tables XV and XVI do not agree with Table I, because this table includes most of the fires on private land in and adjacent to the forests, on which data were available. On the other hand, Table I includes some fires not included here, because elapsed times were not given.

TABLE XVI.—*Number of fires by elapsed time groups, for special risk fires in all types*

Region	Total number of fires	Fires by hours elapsed from discovery to start of suppression work												
		Less than 1	1 to 2	2 to 3	3 to 4	4 to 5	5 to 6	6 to 7	7 to 8	8 to 9	9 to 10	10 to 15	15 to 20	More than 20
1	115	108	3	2	1							1		
2	337	291	24	3	4	2	1	1	2	1	4	1		3
3	202	153	20	6	5	1	3	2			2	7	2	1
4	42	22	6	2	1	2		3	2			2	1	1
5	24	17			1		2					1	1	2
6	82	66	8	2	2	1				1			2	
7	53	39	9	2	2	1								
8	24	17	3		2	1	1							
9	33	24	7	1		1								
10	33	14	4	3	2	1	1				2	3	1	2
11	175	131	25	9	3	2	2	1		1		1		
12	148	120	15	5	2	1	1		1		1	2		
13	91	62	16	7								2	3	1
14	8	4		1				1		1				1
15	12	5	2	2	1	2								
16	214	155	44	6	1	1	3		1			1		2
17	113	86	15	2	3		1	1	2	1	1	1		
18	155	138	13	1			1		1			1		
19	119	71	29	8	4	2		2		1		1		1
20	17	7	4	3	2									1
21	180	151	15	5	2	1		1	1		2	2		
Total [a]	2,177	1,681	262	70	38	19	16	12	10	6	12	26	10	15
Per cent	100	77.2	12.0	3.2	1.7	0.9	0.7	0.6	0.5	0.3	0.6	1.2	0.5	0.7

[a] See note (a), Table XV.

PRIVATE LANDS

For purposes of rating liabilities, no consideration has been given to values on private lands within or adjacent to forest boundaries, because the cost of protecting such values should be met by the owners. It may in some cases be desirable to protect timber values on private lands, because of the possibility of the land being acquired later by the public, through exchange or in some other way. In such cases, arrangements should be made whereby the owner pays the cost of protection either now or at the time when the land is transferred.

In computing special liabilities for any forest units, due allowance should of course be made for special risk fires originating on adjacent or included lands which if not controlled may spread to the protected lands. Fires starting on a railroad right of way running through a forest, for instance, are as much a source of danger to the forest as if the right of way were owned by the public. Where, however, the right of way is so isolated by fire lines or otherwise that no fires ever have escaped from it on to the protected lands, fires occurring on the railroad land would not be counted in computing the liability.

FIRE PLAN RECONNAISSANCE

To apply the method of rating liabilities outlined in the preceding pages, or any other method, for that matter, it will be necessary to make some kind of a survey of the lands and resources that are to be protected. For a preliminary rating, this can be done in a rather extensive way, without a great amount of detailed field work, but such a rating should be followed, eventually, by a more accurate and detailed one based upon an intensive survey. In the meantime, more accurate and complete records should be kept of all fires that occur, in order to afford a better basis for rating liabilities.

DATA TO BE OBTAINED

The survey, whether extensive or intensive, should result in the following information for each forest and protection unit (or ranger district):

1. A map, showing the location and extent of all areas of each class of risk, together with the hour control effective for each area, under the existing conditions. The amount of detail used in classification of risks will depend upon the intensity of the survey.

2. Statistical data regarding the areas subject to risk, classified as shown on the map. These data may be represented in tabular form, somewhat as follows:

A.—AREAS SUBJECT TO GENERAL RISK

Unit	Forest type	Age class	Risk class	Hour control			Quantity and value of timber and young growth		General liability factor	Total general liability
				Less than hour (acres)	1 to 2 hours (acres)	Etc. (acres)	Total	Per acre		

"General liability factors" are the factors for total liability per 1,000 acres, as already given in Table XII. With the data so far available there will be one uniform factor for each type in a given region, regardless of its age or risk class, but varying with hour control. When more detailed ratings can be made, it will be desirable to use different liability factors for stands of different ages and different relative risk. It will also be desirable to take into account the differences in quantities and values at stake, possibly by expressing liability of loss in terms of percentage of total values, instead of directly in dollars.

The preliminary reconnaissance need not show age classes or risk classes, since we have not the data necessary to rate them separately. It is obvious, however, that both costs of suppression and amounts of damage will vary rather widely with differences in age or risk class within a single type, and separate ratings should be made as soon as fire records with the necessary basic information accumulate. It is desirable to have information regarding the distribution of age classes and risk classes for use in planning protection, even though we can not yet rate liabilities in such detail. The classification as to hour control should be based on what is reasonably possible with the existing or proposed protective organization, taking into consideration location of personnel and means and speed of travel.

3. Special information should be given regarding areas of high liability, whether due to probability of occurrence of fires, to probability of rapid spread and of difficulty in suppression, or to probability of heavy damage because of the size of the area likely to burn over, or because of high destructible values. Such information should describe the reasons for the high liability, and, if possible, suggest means of reducing it.

SPECIFICATIONS FOR CLASSIFYING RISKS

The following specifications are suggested as a basis for the classification of areas indicated in the maps and tables described above. The limit of subdivision should be approximately 40 acres for the intensive survey, or 160 acres for an extensive one; or, in other words, no area should be distinguished on the map, or counted in preparing the tables, unless it is at least 40 or 160 acres, respectively, in extent. Smaller areas should be thrown with the neighboring ones. Exceptions to this rule may be made in the case of smaller areas of especially great liability.

FOREST TYPES.—To be classified on the basis of present cover, because that is what is being protected and what determines the hazard and liability. All areas which are fairly satisfactorily stocked with tree growth, no matter how small, should be classed with the

B.—AREAS SUBJECT TO SPECIAL RISKS

Number of fires per year per unit area of the type, for the given unit and cause [a]	Special liability factor	Total special liability (for this cause)

[a] The same data for each kind of special risk separately, as in A, except for the last two columns, for which should be substituted the last two columns of B.

type represented by those trees, and not with the type represented by the possibly more obvious cover, such as brush. In other words, brushfields which have a good stand of tree reproduction beneath the brush cover should be classed with the proper timber type, and not with "brush." In case of two-storied types, as conifers under aspen in some parts of the Great Basin region, the cover should be classed according to the species of chief economic or silvicultural importance. For instance, if such a stand is to be handled as an aspen forest, the conifers may be considered as underbrush, and the type be called "aspen." But if the conifers are to grow to maturity and become the chief crop, and the aspen represents only a temporary phase of the development of a conifer stand, then the cover should be designated as belonging to the proper conifer type. Strictly speaking, there should be classed as "subalpine" only the strictly noncommercial scrubby or scattered high altitude stands, although the ratings as developed in this study undoubtedly included some merchantable fir and spruce and probably some lodgepole pine stands as subalpine.

In general, the definitions of the different types will be about the same as those prescribed for use in timber surveys. In some cases, however, two or more of these types have been grouped together in the present study, and some of these groups may be allowed to stand even in working out more detailed ratings in the future. Others should be separated if possible. Such are the aspen type of the Central Rocky Mountains, now combined with other types; the Engelmann spruce and subalpine types, now combined in several regions; and the brush, grass, and woodland types, now thrown together in a number of cases. In some instances, where a type occurs over a limited area within a region, it has been combined with other types. Thus the limited areas of western yellow pine in the Northern Rocky Mountain region should be thrown in with the Douglas fir or lodgepole pine types.

AGE CLASSES.—Classification as to age should be based on the age of the major part of the stand; for instance, a very scattered stand of old seed trees, over a fairly well-stocked stand of reproduction, would be classed as reproduction; a stand composed of trees of several age classes, but with a large preponderance of mature trees, would be classed as mature. Not more than five age-classes should be recognized. These are:

1. Reproduction	Trees up to 4 inches d. b. h.
2. Small poles	Trees between 4 and 7 inches d. b. h.
3. Large poles	Trees between 8 and 11 inches d. b. h.
4. Young merchantable.	Trees 12 inches or more in d. b. h., up to the rotation age, or the age generally considered as representing maturity.
5. Mature and overmature.	

In addition to these five classes, a sixth class of stand should be recognized, viz: All-aged, where practically all ages are present in approximately equivalent proportions.

RISK CLASSES.—Each stand, after being classified according to type and age class, should be further classified according to the degree of risk involved. "Risk" is used here in the sense of inflammability and controllability, independent of the probability of fires starting or of the presence or absence of a protective organization. For the purpose of rating liabilities, three risk classes should be recognized, based on the susceptibility of the stand to fire. This susceptibility is determined by the fire resistance or inflammability of the component species and of the ground cover, and to some extent by topographic conditions, which favor or hinder rapid spread and destructiveness of fires, and make control work difficult or easy. These classes may be designated as low risk, average risk, and high risk, and should represent the relative risks as between stands of the same type and age class, but not between stands of different types. A stand of western yellow pine classed as "high risk" might not represent as great damage or cost as a "low risk" stand of western white pine, but it would represent a risk greater than the average for the yellow pine stands in the region concerned. Brief tentative specifications for the different risk classes follow.

LOW RISK

Reproduction.—Young trees scattered as individuals or in patches, with comparatively little brush or litter, or where the cover is grazed fairly close before the fire season, or where the inflammable ground cover as a whole—including tree reproduction, grass, weeds, brush, and litter—is not continuous, but is broken by numerous openings or patches of bare soil, rock, or less inflammable vegetation, (such as bear clover). On sheltered flats or moist bottoms, the cover may be more continuous.

Pole stands.—For larger poles, stands with comparatively little undergrowth or dead and down material, and with boles fairly clear of dead branches or moss. For smaller sizes of poles, broken stands with noninflammable openings. For all sizes, stands on sheltered flats and in moist situations.

Merchantable stands.—Comparatively open stands with clear boles, little undergrowth except grass and weeds, or tree reproduction less than 1 foot high, with few standing snags and little litter or débris. Trees not badly scarred at their bases, nor covered with dry moss or pitch. In mixed types, stands composed largely of the more fire resistant species of the mixture. In all-aged stands, those where older trees largely predominate. No deep continuous layer of duff. Stands on sheltered flats and on moist sites which in other situations might fall in a more hazardous class.

AVERAGE RISK

Reproduction.—Stands of fair density, with a fairly continuous cover of light herbage and scattered brush, with only a moderate amount of scattered débris, or with considerable litter entirely shaded and kept from drying out by a dense crown cover. Such stands on moist flats might be classed as low risk, and on steep slopes exposed to drying winds as high risk.

Pole stands.—Larger sizes, with some undergrowth and débris, or with average amount of dry lower branches. Smaller sizes, with comparatively little, or only patchy, inflammable ground cover.

Merchantable stands.—Stands with a fair amount of undergrowth, including tree reproduction, and with more or less débris, scattered standing snags, and more moss, low crowns, or dry lower branches. Stands with average proportions of the more inflammable species.

HIGH RISK

Reproduction.—Either open or dense stands, with heavy grass, dry during the fire season, or a continuous cover of brush and débris to carry fire. Stands on steep slopes and other sites exposed to drying winds. An extreme example of a "high risk" stand of reproduction or poles is found in the "jack-straw" burns common in many regions.

Pole stands.—The larger sizes, where there are large amounts of inflammable ground cover and débris, moss on stems, low inflammable crowns, standing snags, or on steep exposed sites. In mixed stands those with larger proportions of the less resistant species. The smaller poles, where there is a continuous cover of brush or inflammable débris, even if not especially great in quantity. Stands on steep slopes or most exposed situations, which on other sites might fall into the "average risk" class.

Merchantable stands.—Stands with large amounts of inflammable undergrowth or débris (such as logging slash), large numbers of standing snags, bases of trees badly fire-scarred, boles covered with dry branches or much dry moss or resinous bark. Stands with somewhat less inflammable material, where especially exposed to drying winds or on steep slopes. In mixed stands, those where the least resistant species are represented in large numbers; in all aged stands, those with a large proportion of the younger ages. Stands with a deep layer of duff or peat which dries out during the fire season.

DESTRUCTIBLE VALUES

Values should be tabulated as indicated, for individual stands. These values represent what would be lost in case of total destruction by fire, and include values of timber, both merchantable and young growth, of the forest capital (not including soil productivity), where that is involved, and of intangibles such as watershed protection. Forage value is omitted, unless it can be considered to be included in the figures given for the other values, for the reason already mentioned, viz, that destructible value of forage is generally so insignificant in comparison with the other values as to be less than the probable error in estimating the others. For the sake of simplifying calculations, therefore, it is left out.

In order that such values can be easily gotten at, and to insure that the values given shall be comparable as between different forest units and between different regions, and also in order that figures on damage during a series of years may be computed on the same basis and may therefore be possible of comparison—which is not the case with the records hitherto collected—it seems extremely desirable to establish standards, to be used uniformly, without the necessity of leaving very much to the individual judgments or guesses of reporting officers.

These standards should be as simple as is consistent with the purpose for which they are to be used, namely, to show relative values and relative damages as between stands of different species, of different ages, and in different regions. Moreover, they should be in such form as to enable the field men to work the values out without mathematical formulae. With these ideas in mind, standard values were worked out as outlined below.

VALUES OF MERCHANTABLE TIMBER

Merchantable timber, i.e., timber of merchantable size in "young merchantable," "mature," and "all-age" stands, should be valued on the basis of its species and volume, at fixed stumpage rates. For reasons already discussed, it seems advisable to use uniform rates for one species throughout a given region The values suggested have been given in Table VIII. Such scattered trees of merchantable size as may be found in "reproduction" and "pole" stands should not be given a timber value, because they generally would not be utilized for timber. No additional allowance is made for the capital value of merchantable stands, because, in general, natural reproduction will follow the destruction of such stands, if further fires and grazing are kept out. No value will be put on the timber, as such, in scrubby high-altitude subalpine stands that never will be utilized for timber production. Their value consists entirely of the intangible values for watershed protection and the like, and for mature stands will be the same as given in Table XVII for "large poles."

VALUES OF YOUNG GROWTH

The timber value of young growth depends upon its species and the stage of development which it has reached. This value is the value of the accrued net return on the capital value of the forest, for a number of years equal to the economic age of the stand. For natural stands, such as practically all of those on the national forests, it can be expressed by the formula:

$$V = Y \times \frac{1.0p^m - 1}{1.0p^n - 1}$$

Here Y is the value of the final crop (for the sake of simplicity no allowance is made for intermediate returns from thinnings), $.0p$ is 3 per cent, m is the economic age of the stand, and n is the number of years in the rotation.

The value of Y depends upon the stumpage value per thousand board feet and the amount of timber that will be produced during a rotation. For the purpose of establishing standard values for young growth, arbitrary rotations and yields were used, with the stumpage rates given in Table VIII. Because we know very little about what rotations will actually be used for stands now below merchantable size, it seemed advisable for the present purpose to use a uniform rotation period of 100 years, regardless of forest type, in the regions where growth is moderately fast (regions 2, 3, 4, 5, 6, 7, 8, 16, and 17), and 150 years where it is slower (regions 1, 9, 10, 11, 12, 13, 14, 15, 18, 19, and 20). For the Lake States (region 21) a 70-year rotation was used.

In order that the field men may not have to estimate the ages of the stands, and to reduce the division into age classes to the simplest practicable terms, only three age classes of young growth are recognized, and these are expressed in terms of size, rather than of age. They are: Reproduction (stands below 4 inches d. b. h.); small poles (4 to 7 inches d. b. h.); and large poles (8 to 11 inches d. b. h.). In stands classed as "all-aged," young growth of all sizes will be lumped together. For computing values by the formula, the average ages of these size-classes were assumed to be: For 150-year rotation, 20, 40, and 70 years, respectively; for 100-year rotation, 15, 30, and 50 years; and for 70-year rotation, 10, 20, and 35 years.

Besides the timber, or product, value of young stands, there is their value as part of the forest capital. The destructible part of this value (aside from soil productivity, which will not be considered) is the cost of establishment. That is to say, when a new stand has been established on the burned area, the forest capital (but not the accumulated product) has been restored. If a destroyed stand is quickly replaced by natural reproduction, it is considered that no capital loss has been suffered; but where natural restocking does not follow, the capital loss, equal to cost of replanting, must be added to the product loss. Standard costs of planting have been given in Table IX. For the sake of simplicity and uniformity, it has been assumed that stands of young growth covering less than 10 acres, and also young growth of any area under merchantable stands or scattered through all-aged stands, will be replaced by seeding from the sides or from above if destroyed by fire, and that areas greater than 10 acres (except where mixed with older timber) will not restock naturally. Burns smaller than 10 acres, within more extensive stands of young growth, can not be depended upon to restock naturally within a reasonably short period; therefore damage in such cases will include the capital loss. Exceptions to the above assumption are: the western yellow pine in regions 14, 15, 18, 19, 20, where it was assumed that only one-half of the destroyed area will restock even in stands smaller than 10 acres; Douglas fir in the same regions, where it was assumed that only two-thirds of the destroyed area will restock in stands smaller than 10 acres; the woodland types in all regions, where only one-half will restock in stands under 10 acres in extent; lodgepole pine and jack pine stands of the "large pole" class, where it was assumed that half the burns will restock naturally even in stands larger than 10 acres; and the hardwood and aspen types in all regions, which will restock entirely, even on large burns.

INTANGIBLE VALUES

There appear to be no data upon which to base valuations of watershed protection, recreation values, or other similarly intangible benefits due to the presence of a forest cover, or to estimate what part of such values might be destroyed by a fire. It seems fairly reasonable to suppose that in most cases where reproduction will follow naturally almost immediately after the burn, the damage to such values is comparatively small; where such restocking will not take place, the damage estimates

already allow for the cost of restoring a stand by planting, which is probably considerably greater than the actual intangible loss. No additional value, therefore, has been allowed for "intangibles," except in the case of brushland and grassland, and subalpine areas that do not produce merchantable timber, and the southern California hardwood bottomlands type, that has small commercial value and a very great intangible value. For these types, arbitrary intangible values were assumed. Except in the case of the Southern California stream bottom type, it was also assumed that very little or no damage would be done to the intangible values by fires covering less than 40 acres. In this type, even small fires, if they destroy the cover, do considerable damage, even though natural restocking quickly follows, and the damage where stands of mature trees are destroyed is at least as great as for younger stands, regardless of the timber value.

TOTAL VALUES

The standard total destructible values per fully stocked acre of young growth of the different types and in the several regions have been given in Table XVII. For the use of field officers within a given region, these values are not as complicated as they may appear here, because any one officer will have to consider only the figures that apply to his particular region, and to the types which are found on his particular district.

TABLE XVII.—*Standard total destructible values of young growth*

Type	Region	Destructible value per well-stocked acre						
		Reproduction		Small poles		Large poles		Reproduction and poles in all aged stands
		Areas under 10 acres	10 acres and over	Under 10 acres	10 acres and over	Under 10 acres	10 acres and over	
		Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
Yellow pine, including western yellow pine-sugar pine mixtures in California.	1,9,10,11	0.60	9.10	1.65	10.15	5.00	13.50	2.00
	2	1.25	8.25	3.15	10.15	7.40	14.40	2.70
	3	1.40	7.40	3.50	9.50	8.35	14.35	3.00
	4	1.55	11.55	3.90	13.90	9.30	19.30	3.30
	6	2.40	12.40	6.10	16.10	14.50	24.50	5.20
	7	1.65	11.65	4.25	14.25	10.00	20.00	3.60
	8	1.45	11.45	3.75	13.75	8.90	18.90	3.20
	12, 13	.45	12.45	1.20	13.20	3.65	15.65	1.50
	14, 15	7.75	15.25	8.20	15.70	9.60	17.10	8.30
	16	2.75	14.75	7.05	19.05	16.70	28.70	6.00
	17	9.05	16.55	11.40	18.90	16.80	24.30	10.80
	18, 19	8.00	15.50	8.85	16.35	11.65	19.15	9.25
	20	8.10	15.60	9.15	16.65	12.50	20.00	9.50
Douglas fir, including western larch mixtures in the northwest, lower slope mixture on west coast, and bigcone spruce slopes in southern California.	1, 12, 13	.20	10.20	.60	10.60	1.90	11.90	.75
	2	1.05	7.05	2.75	8.75	6.60	12.60	2.30
	3	1.40	9.40	3.50	11.50	8.35	16.35	3.00
	4	1.40	11.40	3.50	13.50	8.35	18.35	3.00
	5	2.75	10.25	7.00	14.50	16.70	24.20	6.00
	6	2.55	10.05	6.55	14.05	15.50	23.00	5.60
	7, 8	.85	10.85	2.20	12.20	6.00	16.00	1.90
	9	.25	10.25	.70	10.70	2.20	12.20	.90
	10	.30	10.30	.80	10.80	2.50	12.50	1.00
	14, 15	5.30	15.30	5.80	15.80	7.50	17.50	6.00
	16	1.40	11.40	3.50	13.50	8.35	18.35	3.00
	17	10.90	20.90	12.35	22.35	15.60	25.60	12.00
	18, 19, 20	4.40	12.40	5.00	13.00	7.15	15.15	5.25
Lodgepole pine, including knobcone pine in California.	1, 12	.35	10.35	.95	10.95	2.95	7.95	1.20
	2, 16	.90	8.90	2.35	10.35	5.60	9.60	2.00
	3, 6	1.55	9.55	3.90	11.90	9.30	13.30	3.30
	4	1.05	11.05	2.75	12.75	6.60	11.60	2.30
	5	1.20	9.20	2.90	10.90	7.00	11.00	2.50
	7	1.40	11.40	3.40	13.40	8.15	13.15	2.90
	8	1.25	11.25	3.15	13.15	7.40	12.40	2.70
	9	.30	10.30	.85	10.85	2.70	7.70	1.10
	10, 13, 14, 15, 19	.40	10.40	1.05	11.05	3.30	8.30	1.30

TABLE XVII.—*Standard total destructible values of young growth*—Continued

Type	Region	Destructible value per well-stocked acre						
		Reproduction		Small poles		Large poles		Reproduction and poles in all aged stands
		Areas under 10 acres	10 acres and over	Under 10 acres	10 acres and over	Under 10 acres	10 acres and over	
		Dolls.	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*	*Dolls.*
Spruce and fir (not subalpine), including cedars and hemlock when not included in white pine or Douglas fir types.	1, 9	0.20	6.70	0.50	7.00	1.50	8.00	0.60
	2	.90	6.90	2.35	8.35	5.60	11.60	2.00
	3	1.40	7.40	3.50	9.50	8.35	14.35	3.00
	4	1.20	8.70	2.90	10.40	7.00	14.50	2.50
	5	1.25	8.75	3.15	10.65	7.40	14.90	2.70
	6	.90	8.40	2.35	9.85	5.60	13.10	2.00
	7	.70	8.20	1.75	9.25	4.20	11.70	1.50
	8	.50	8.00	1.25	8.75	3.00	10.50	1.10
	10	.30	6.80	.80	7.30	2.50	9.00	1.00
	12	.25	10.25	.70	10.70	2.20	12.20	.90
	13, 15	.20	10.20	.60	10.60	1.90	11.90	.75
	14	.25	10.25	.65	10.65	2.00	12.00	.80
	16	1.40	9.40	3.50	11.50	8.35	16.35	3.00
	18, 19, 20	.30	10.30	.90	10.90	2.85	12.85	1.10
	21	2.00	8.00	4.65	10.65	10.50	16.50	4.00
Western white pine	2	3.50	8.50	8.90	13.90	21.10	26.10	7.60
	3	4.50	10.50	11.45	17.45	27.15	33.15	9.75
Eastern white pine and Norway pine.	21	10.20	16.20	26.20	32.20	59.00	65.00	22.50
Jack pine	21	1.50	11.50	3.50	13.50	7.85	12.85	3.00
Hardwoods	17	20.00	20.00	20.00	20.00	20.00	20.00	5.00
	21	2.50	2.50	5.80	5.80	13.10	13.10	5.00
Subalpine, noncommercial high altitude type.	1, 9, 10, 12, 13, 14, 15, 18, 19, 20.	.....	10.10	.....	10.50	.....	11.00	.25
	4, 5, 6, 7, 8, 16.	.....	8.10	.....	8.50	.....	9.00	.25
	2, 3	.....	6.10	.....	6.50	.....	7.00	.25
	17	.....	15.10	.....	15.50	.....	16.00	.25
Aspen	All regions where of commercial value.	.60	.60	1.60	1.60	3.70	3.70	1.30
Woodland. Includes piñon-juniper, digger pine-oak, and similar types.	4, 7, 8	2.60	5.10	2.80	5.30	3.20	5.70	2.75
	6, 12, 13, 14, 15, 17, 18, 19, 20.	2.75	5.25	3.10	5.60	3.90	6.40	3.00
	16	2.95	5.45	3.70	6.20	5.30	7.80	3.50
Brush, not restocking with commercial species. Includes chaparral type and other brushfields.	1, 2, 3, 5, 9, 10, 11, 12, 13.	Uniform value $0.10 per acre (for areas greater than 40 acres).						
	4, 6, 7, 8, 16.	Uniform value $0.25 per acre (for areas greater than 40 acres).						
	14, 15, 18, 19.	Uniform value $0.50 per acre (for areas greater than 40 acres).						
	17	Uniform value $5.00 per acre (for areas greater than 40 acres).						
	20	Uniform value $2.00 per acre (for areas greater than 40 acres).						
Sagebrush and grassland	All regions.	Uniform value $0.05 per acre.						

FIRE RECORDS

To provide an accurate basis for future rating by the methods outlined in this report, or by any other scientific method, it is essential that accurate records be kept of all fires throughout the country. Since the value of such records varies directly with their completeness and accuracy—inaccurate records are but little more useful than none at all—it will be decidedly worth while to take considerable pains to see that they are made and kept in good shape. All reports on individual fires should be checked up by a competent supervisory officer to see that they give the information that is required to make them useful. For the purpose of rating hazards and liabilities, the reports for each protective organization should always give at least the following information; other data may be desired from time to time for administrative studies of various sorts:

1. Location of the fire.
2. Date and hour of the discovery of the fire (and of its start if known); of the start of work on it; when it was under control; when it was out.
3. Cause, in detail. For instance, if a camper fire, what kind of a camper—traveler, sheep-herder, campfire?
4. Cover. Forest type, age class, risk class.
5. Area burned, classified according to types, age, and risk classes, if more than one.
6. Destructible values on the burned area before the fire.
7. Losses—quantities (thousand feet by species, and fully stocked acres of young growth by types and age classes, if more than one) and values, according to standard figures.
8. Costs of suppression—itemized in such a way that that part of the cost chargeable to primary protection may be kept distinct from special fire-fighting costs.

In addition to the detailed individual reports on all fires, which may be transferred to tabulation sheets or punched cards for convenience in filing and future study, it is also desirable that sets of maps be kept up to date showing the character and values of the cover on the whole area, and the locations of all fires covered in the reports. Such maps should be on a fairly large scale, preferably one-half inch or one inch to the mile. The fire records should be made permanent, and those for any one organization should preferably be kept all together in one place.

ASCARIDIA LINEATA, A PARASITE OF CHICKENS IN THE UNITED STATES[1]

By BENJAMIN SCHWARTZ

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INTRODUCTION

Heretofore it has been assumed that the large intestinal roundworm of chickens in the United States is *Ascaridia perspicillum*, a species described from the intestine of turkeys by Rudolphi in 1803 (*12*).[2] A recent study by the present writer of specimens of Ascaridia collected at various times from the intestine of chickens, in and around Washington, D. C., in New Jersey, Kansas, and in several other localities, has shown that the species in question does not conform to the description of *A. perspicillum* as given by various writers, but conforms in practically all respects to the description of another species, namely, *A. lineata*, which was described by Schneider in 1866 (*13*).

These two species have undoubtedly been confused in the past, partly owing to their close relationship, and partly because the tendency of most workers who are not systematists is to make host determinations of parasites. The fact that *A. perspicillum* is an earlier described species and that its type locality is Europe is probably largely responsible for the importance that has been assigned to it in textbooks on parasitology, whereas *A. lineata*, which was originally described from Brazil, has received but secondary consideration in the widely used textbooks on parasitology, nearly all of which have been written by European workers. As will be presently shown, *A. lineata* is not only more prevalent then *A. perspicillum* in the United States and in all probability in other parts of the world, but is also possibly the only species of Ascaridia parasitic in chickens in this country, the occurrence of *A. perspicillum* in American chickens not yet having been definitely established.

ASCARIDIA PERSPICILLUM (RUDOLPHI, 1803)

Rudolphi's description of *Ascaris perspicillum* is based on immature female specimens from the turkey, and, in common with specific diagnoses of a century ago, contains little that is of diagnostic value. According to Dujardin (*3*) *A. perspicillum* of Rudolphi is apparently identical with *Fusaria inflexa* of Zeder. Dujardin (*3*) states that the entries in the catalogue of the Vienna Museum, whose helminthological collections were studied by Rudolphi, contain no reference to *A. inflexa* from chickens, this species being recorded only from ducks. Dujardin also states that the catalogue in question contains but a single entry of *A. perspicillum* from the turkey.

Schneider (*13*), in his extensive monograph on nematodes, states that he examined one male and one female specimen of *Heterakis perspicillum* and found them to be identical with *H. inflexa*, of which he also examined specimens. In both cases the material examined appears to have been material studied by Rudolphi. Although Schneider was unable to make out the structure of the lips in the specimens labeled *Ascaris perspicillum*, he had no difficulty in making out the papillae on the tail of the male, which he found to correspond to those of *A. inflexa*. Since the papillae on the tail of the male are commonly regarded as the most important specific characters in the suckered roundworms (Heterakidae), it may be taken for granted that Schneider was correct in regarding *A. perspicillum* and *A. inflexa* as identical.

Schneider figures 9 pairs of papillae on the tail of the male of *Heterakis inflexa* (fig. 1), arranged as follows: 3 pairs of ventral papillae arranged in a row on each side of the sucker, the most cephalad papillae being anterior to, and the third, or most caudal pair, being posterior to the sucker, whereas the middle pair corresponds in position approximately to the equator of the sucker. The next group consists of 4 pairs of papillae, of which 3 pairs are lateral and 1 pair is ventral, and finally, there are 2 pairs of lateral papillae near the tip of the tail.

[1] Received for publication June 30, 1924; issued June, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 772.

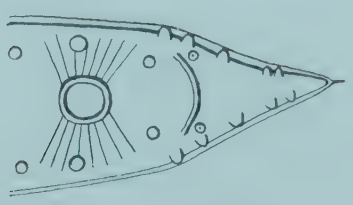

FIG. 1.—*Heterakis inflexa* (after Schneider, 1866)

The views expressed by Dujardin (*3*) and Schneider (*13*) as regards the specific indentity of *Ascaridia inflexa* and *A. perspicillum* have been generally accepted by helminthologists, including Railliet (*10*), who states that this species has 10 pairs of papillae, but makes no statement as to their arrangement, excepting that 3 pairs are preanal and 7 pairs are postanal. Railliet and Henry (*11*), in their paper on the classification of the Heterakidae, list *A. perspicillum* as being possibly identical with *A. inflexa*, a similar attitude of uncertainty being expressed by Skrjabin (*14*).

Recently *Ascaridia perspicillum* has been figured by Baylis and Daubney (*1*) (fig. 2) and by Smit (*15*) (fig. 3). According to the former, there are 10 pairs of papillae on the tail of the male, the first group consisting of 4 pairs of ventral papillae, instead of 3 pairs as figured by Schneider, the remaining papillae agreeing as to number and position with those of Schneider. Smit, on the other hand, while agreeing with Baylis and Daubney as to the number of papillae, shows a somewhat different mode of arrangement, since he figures 2 ventral and 3 lateral pairs of papillae in the second group, as compared with 3 pairs of lateral and only 1 pair of ventral as figured by Schneider and by Baylis and Daubney. The remaining papillae as figured by Smit correspond to those figured by Schneider.

The different conceptions as to the number and arrangement of the papillae of *Ascaridia perspicillum* may be summarized as follows:

	Schneider	Baylis and Daubney	Smit
Group 1	3 pairs of ventral papillae	4 pairs of ventral papillae	3 pairs of ventral papillae.
Group 2	3 pairs of lateral papillae	3 pairs of lateral papillae	3 pairs of lateral papillae.
Group 3	1 pair of ventral papillae	1 pair of ventral papillae	2 pairs of ventral papillae.
	2 pairs of lateral papillae	2 pairs of lateral papillae	2 pairs of lateral papillae.

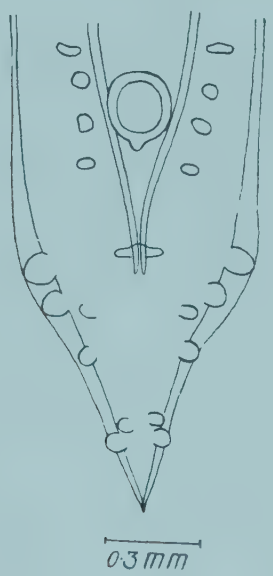

FIG. 2.—*Ascaridia perspicillum.* (After Baylis and Daubney, 1922)

Assuming that Schneider, Baylis and Daubney, and Smit were dealing with the same species, it will be seen from the comparison of their descriptions of the papillae that they all agree as to the presence of only two lateral papillae in the region of the tip of the tail, the number of ventral papillae in the anterior two groups showing variations. Whether the species in question (*As-*

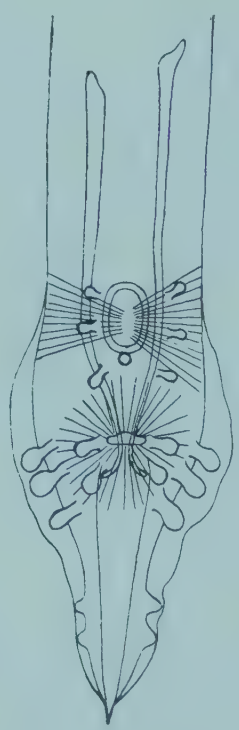

FIG. 3.—*Ascaridia perspicillum.* (After Smit, 1922)

caridia perspicillum) is variable with respect to the ventral papillae, or whether under the name *Ascaridia perspicillum* two or more species have been confused, must be left an open question. So far as concerns the arrangement of the papillae on the tail of the male in specimens of *Ascaridia* from chickens in the United States and from other localities that have been examined by the present writer, they do not correspond to any of the patterns described for *A. perspicillum*, but agree with that of another species, namely, *A. lineata*.

ASCARIDIA LINEATA (SCHNEIDER, 1866)

Schneider (*13*) describes *Heterakis lineata* from the intestine of the chicken from Brazil in the same paper in which he discusses the identity of *Heterakis perspicillum* and *H. inflexa*. Schneider differentiates *H. inflexa* (*H. perspicillum*) from *H. lineata* on the basis of the structure of the lips as well as on the basis of the number of papillae on the tail of the male. He states that *H. inflexa* has 3 dentigerous ridges on each lip, the first 2 being round and the third (most posterior) being quadrangular, whereas *H. lineata*, according to this writer, has only 2 dentigerous ridges, the second ridge being very small. As far as concerns the papillae on the tail of the male, Schneider figures 10 papillae for *H. lineata* (fig. 4), the last group of papillae in the region of the tip of the tail consisting of 3 pairs, 2 lateral and 1 ventral, as compared with only 2 pairs of lateral papillae in *H. inflexa*. The remaining papillae, according to Schneider's figure, correspond in number and position to those of *H. inflexa*.

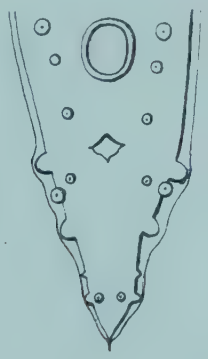

FIG. 4.—*Heterakis lineata* (after Schneider, 1866)

Other records of the occurrence of *Ascaridia lineata* besides those given by the present writer are as follows:

Von Linstow (*7*) records and figures *Heterakis lineata* from the duck from Turkestan. The number and arrangement of the papillae as figured by Von Linstow correspond accurately with those of Schneider, although there are two discrepancies, namely, as regards the elongation of the first ventral papilla, which is figured as transversely flattened by Von Linstow (fig. 5), whereas Schneider figures it as spherical in shape. As regards the second lateral papilla, Von Linstow figures it as directed laterally, whereas Schneider figures it as directed ventrally.

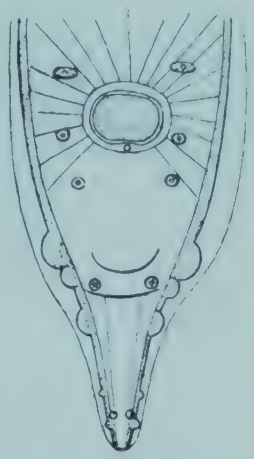

FIG. 5.—*Heterakis lineata* (after Von Linstow, 1883)

Von Linstow (*8*) records *Heterakis inflexa* from the chicken, but from his description of the papillae, in which 3 pairs are enumerated in the region of the tip of the tail, 2 being lateral and 1 ventral, it may be taken for granted that he was dealing with *Ascaridia lineata*.

Wolffhügel (*18*) records *Heterakis lineata* from Switzerland from the chicken and the duck, but he records *H. inflexa* (*Ascaridia perspicillum*) only from the grouse (*Tetrao urogallus*).

Travassos (*16*) records and figures *Ascaridia lineata* from chickens in Brazil, the type locality of this species. His drawing of the tail of the male (fig. 6) shows a perfect agreement with that of Von Linstow (fig. 5) as to number, arrangement, and direction of the papillae.

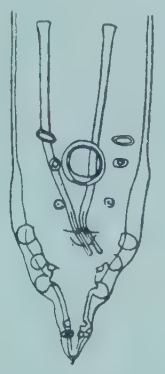

FIG. 6.—*Ascaridia lineata* (after Travassos, 1913)

Gedoelst (*4*) records *Ascaridia lineata* from the chicken in the Belgian Congo.

Boulenger (*2*) records and figures *Ascaridia lineata* from the chicken in Zanzibar on the basis of a single male specimen. His figure (fig. 7) agrees in all respects except one with that of Von Linstow and Travassos, the exception being the direction of the second lateral papilla, which is described as being turned ventrally, thus agreeing in this respect with Schneider's figure. Boulenger observes that these discrepancies are probably due to individual variations.

According to Boulenger (*2*) *Ascaridia hamia*, a species described by Lane (*5*) from the intestine of chickens in Bengal, India, is *Ascaridia lineata*, a view with which the present writer is in full agreement. Lane's figure and description of the papillae (fig. 8) show a perfect agreement with those of Schneider, Von Linstow, Travassos, and Boulenger, and although his description of the species as a whole shows some discrepancies as compared with the present writer's observations on *A. lineata*, these discrepancies can all be accounted for, as will be presently shown, on the basis of individual variation.

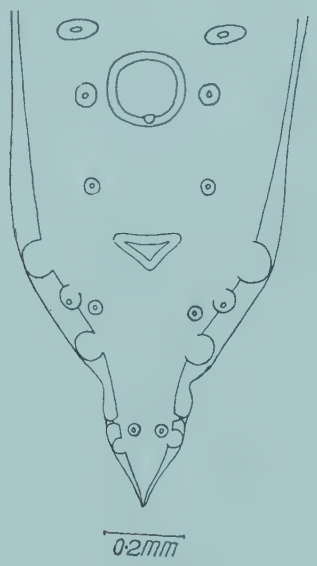

FIG. 7.—*Ascaridia lineata* (after Boulenger, 1923)

According to Travassos (*16*), *Heterakis brasiliensis* Magalhães, 1892 (*9*), is also a synonym of *Ascaridia lineata*. Magalhães' description is rather vague as regards the papillae on the tail of the male and does not agree with his figure in all respects. He states that the worm has 9 pairs of lateral papillae, but he actually enumerates 10 pairs and one accessory papilla. Certain of the papillae referred to by Magalhães as lateral are obviously ventral in position. The description that Magalhães gives of *H. brasiliensis* is too incomplete to permit any definite opinion as regards the status of that species.

In all of the records of the occurrence of *Ascaridia lineata* which have just been reviewed, excepting that of Schneider, no mention is made of *A. perspicillum* as a parasite occurring alone or in association with *A. lineata* in the same host species. This fact is of importance and indicates that when specimens of Ascaridia from chickens and related domestic birds have been actually studied morphologically they have been found to be *A. lineata*, which is apparently the common species of Ascaridia in domestic birds.

FIG. 8.—*Ascaridia hamia* (after Lane, 1914)

OBSERVATIONS ON ASCARIDIA LINEATA BY PRESENT WRITER

Observations on *Ascaridia lineata* by the present writer were made on material collected in the United States by various workers in this laboratory in the vicinity of Washington, D. C., as well as on material forwarded to this bureau for determination from several different localities. In addition to American material, specimens collected from chickens in the Philippine Islands and at Tonkin, Indo-China, were also studied. In material forwarded from Indo-China immature specimens of *A. lineata* from the goose (fig. 9) were identified, this host being new for this species of parasite.

In all specimens that have been examined, the number and arrangement of the papillae on the tail of the male correspond to those described by

various workers for *Ascaridia lineata*. The different variations as regards the first ventral and second lateral papillae which have been referred to elsewhere in this paper were also encountered. The number of papillae on the tail of the male was found to be 10, the first

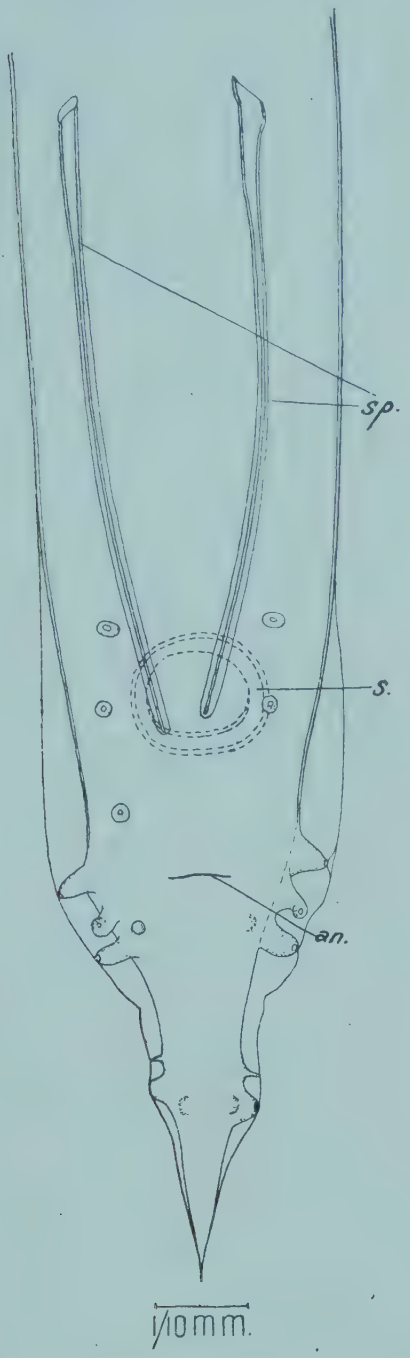

FIG. 9.—*Ascaridia lineata* (original) from the goose. *an.*, anus; *s.*, sucker; *sp.*, spicules

group consisting of 3 pairs (ventral) arranged in a linear series on each side of the sucker, the second group consisting of 4 pairs (3 lateral and 1 ventral), and the last group consisting of 3 pairs (2 lateral and 1 ventral). (figs. 10, 11, 12, 13.)

VARIATIONS IN ASCARIDIA LINEATA

HEAD.—The head varies in size corresponding to different sized specimens. The two subventral lips are practically equal in size, while the dorsal lip is somewhat larger. As has already been stated, Schneider (*13*) described two dentigerous ridges, the second one, according to this worker, being very small. Lane (*5*) refers to only one ridge which

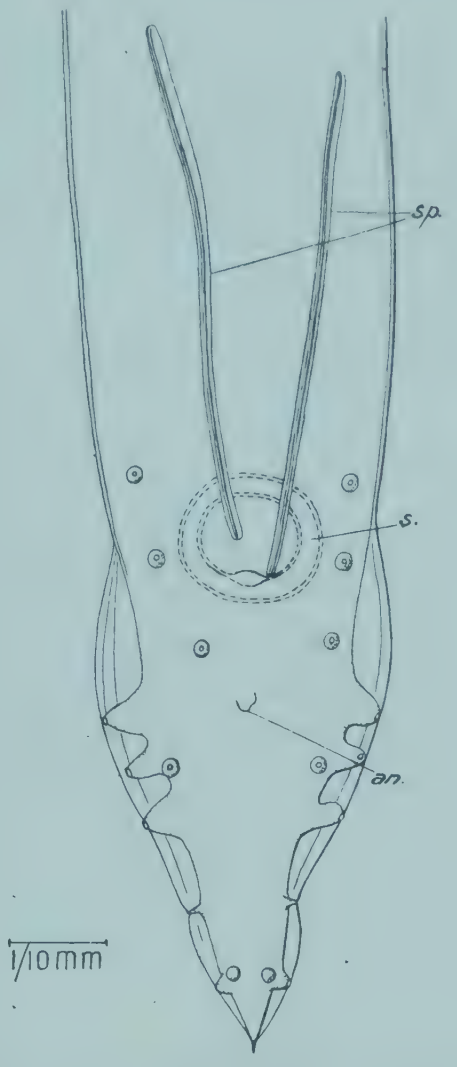

FIG. 10.—*Ascaridia lineata* (original) from the chicken. *an.*, anus; *s.*, sucker; *sp.*, spicules

he states has the appearance of a wavy line when viewed from certain aspects. The present writer observed a rather prominent dentigerous ridge, containing distinct teeth, but failed to make out a second smaller dental plate referred to by Schneider. Each lip bears two papillae, these having been also observed by Lane (*5*). No interlabia are present.

OESOPHAGUS.—In adult specimens the oesophagus is 3 mm. to 4 mm. long, club shaped, and about 0.35 mm. in

maximum width. In immature specimens the oesophagus is relatively shorter and narrower (fig. 14), various gradations in length and width, depending upon the size of different specimens having been observed.

PAPILLAE.—While the number of papillae in the male of *Ascaridia lineata* is constant, the positions of the individual papillae with respect to one another and the direction of the first ventral and second lateral papillae are rather variable (figs. 4 to 13). It has already been pointed out that certain writers describe the first ventral papilla as lying in a transverse direction (figs. 5, 6, and 7), whereas other observers, including Lane (*6*), have figured it as spherical (figs. 4 and 8). The present writer found that in most cases the first ventral papilla is transversely flattened, although in one immature specimen from the goose it was spherical in shape (fig. 9). With regard to the second lateral papilla, it was observed to be directed laterally in all specimens examined, save in one immature form from the goose, in which the papilla was directed ventrally (fig. 9).

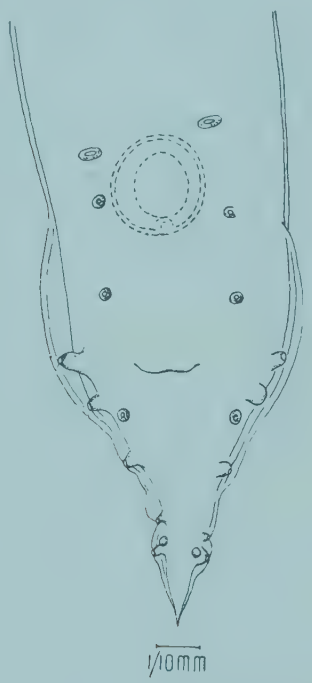

FIG. 11.—*Ascaridia lineata* (original) from the chicken

FIG. 12.—*Ascaridia lineata* (original) from the chicken

Other variations with regard to the papillae are as follows: The last two lateral papillae were found to be very close together in certain specimens and comparatively distant from each other in other specimens. In the middle group of papillae the three lateral papillae were found to be equidistant in some specimens, whereas in other specimens the two anterior papillae of this group were close together and the most posterior papilla relatively distant from the middle papilla. The lateral papillae also show considerable variation in size, relatively large papillae occurring in mature specimens and smaller papillae in very young specimens. The ventral papillae afford the same degree of variation as regards location, shape, and size, the latter variation being correlated with age. The first ventral papilla is considerably anterior to the sucker in certain specimens, whereas in others it is on a level with or somewhat anterior to the cephalad border of the sucker. The fourth ventral papilla is fairly constant in position, somewhat posterior to the

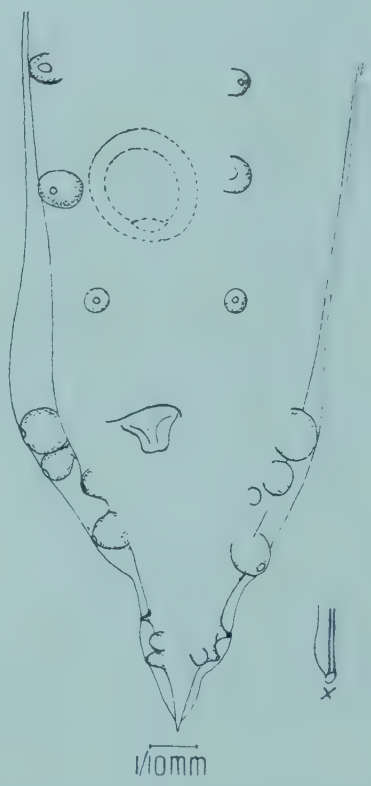

FIG. 13.—*Ascaridia lineata* (original) from the chicken. *x.*, tip of spicule

second lateral papilla, but the location of the last ventral papilla is rather variable, being anterior to the last lateral papilla in certain specimens and posterior to it in other specimens. Not infrequently the arrangement of the papillae on the two sides of the bursa is asymmetrical (fig. 8). The position of the first lateral papilla with respect to the anus is also somewhat variable, being located either slightly anterior to, on a level with, or rarely somewhat posterior to the anus (figs. 4 to 13).

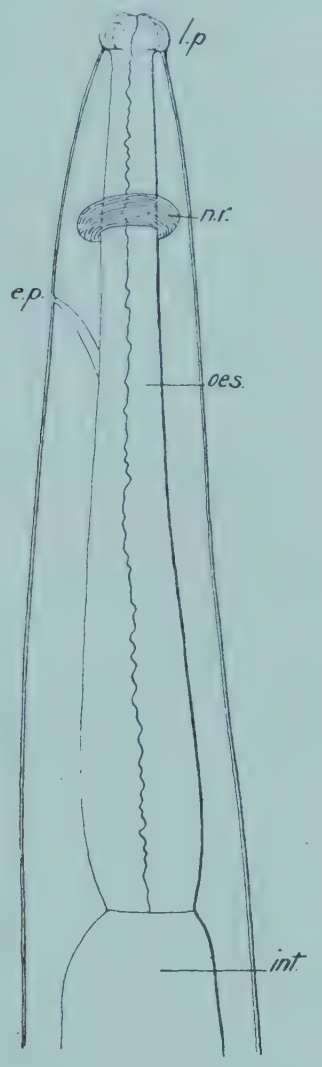

Fig. 14.—Anteria end of *Ascaridia lineata* (original). *e. p.*, excretory pore; *n. r.*, nerve ring; *oes.*, oesophagus.

SPICULES.—A considerable range of variation exists as regards the length of the spicules in *Ascaridia lineata*. According to Travassos (*16*), the spicules are 1.4 mm. long. According to Lane (*5*), they are 2 mm. long. Von Linstow (*8*) states that the spicules of *Heterakis inflexa* (misidentification of *Ascaridia lineata*) are 1.9 mm. long. Immature specimens from the chicken and goose from Tonkin, 1.8 mm. long, showed a range in spicule variation from 0.53 to 0.57 mm. in length. Somewhat larger specimens from chickens had spicules measuring from 0.7 to 0.8 mm. Spicules from not quite fully grown specimens from chickens in the Philippines varied from 0.9 to 1.3 mm. in length. Mature specimens from chickens from Tonkin showed a variation in the length of the spicules from 1.6 to 2.4 mm. Specimens from the United States showed a similar variation in the size of the spicules, the youngest immature forms measured having spicules 0.6 mm. long, whereas large mature forms had spicules somewhat in excess of 2 mm.

In view of the specific importance that is commonly attributed to the size of the spicules in nematodes, the rather wide range of size variation noted in this species is worthy of emphasis.

SUCKER.—The size of the sucker as given by Lane is 0.2 mm. in diameter, agreeing closely with that of Travassos and Boulenger (so far as can be judged from the latter's figure for which a scale is given). The maximum diameter of the sucker as observed by the present writer was 0.25 mm. and the minimum diameter in very young specimens was somewhat less than 0.14 mm

Additional variations were observed as regards the distance of the nerve ring and excretory pore from the cephalic extremity, the length of that portion of the vagina that extends cephalad, and the length of the tail in both sexes. The most conspicuous of these variations are given below: According to Lane (*5*), the vagina runs cephalad for a distance of about 1 mm. before it turns caudad. In very young forms the writer found that the vagina runs cephalad for a distance of only 475 μ (fig. 15), while in somewhat larger specimens that distance was found to be 670 μ. In mature specimens the cephalad portion of the vagina was found to be somewhat longer than that given by Lane. The tail of the male in fully grown specimens was found to be 0.7 mm., as compared with 0.5 mm. given by Lane, with a decrease in size in smaller specimens. The distances of the excretory pore and nerve ring from the cephalic extremity were found to be somewhat greater than those given by Lane in fully grown specimens, but considerably shorter in immature specimens (fig. 14).

The position of the vulva is near the equator according to Lane (*5*), which corresponds to the observations of the present writer. According to Travassos (*16*) it is in the first third of the body. The size of the eggs, which are thick shelled, the shell being smooth, is

about 80 by 50 μ according to observations of the present writer (fig. 16), which is in agreement with the size given by Travassos but is larger than that given by Lane (65×40 μ). Since practically all of Lane's measurements fall short of the present writer's measurements, it may be concluded that no such actual discrepancy as regards the size of the eggs which Lane's figures indicate really exists, the difference being due in all probability to some slight fault in the calculation of measure ments.

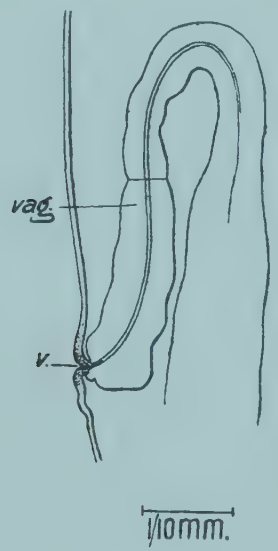

FIG. 15.—Vulva and vagina of *Ascaridia lineata* (original). v., vulva; vag. vagina

Fig. 16.—Eggs of *Ascaridia lineata* (original)

SPECIFIC DIAGNOSIS OF ASCARIDIA LINEATA SCHNEIDER, 1866

Synonyms.—Heterakis perspicillum Von Linstow, 1894 (misdetermination); *Ascaridia hamia* Lane, 1914; *H. braziliensis* Magalhães, 1892 (according to Travassos, 1913).

Mature specimens 70 to 120 mm. long by about 1 mm. or more wide, the females being larger than the males. Lips prominent, each lip provided with two papillae and a dentigerous ridge bearing distinct teeth. Oesophagus simple, from 3 to 4 mm. long by 0.34 mm. wide in adult specimens. The nerve ring is located approximately anterior to the first fourth of the oesophagus and the excretory pore is posterior to the nerve ring.

MALE.—Ten pairs of papillae arranged in 3 groups, as follows: An anterior group of 3 pairs of ventral papillae arranged on each side of the sucker, the middle pair of this group of papillae being alongside, the anterior pair being on the level with or anterior to, and the posterior pair being posterior to the sucker; a second group consisting of 3 pairs of lateral and 1 pair of ventral papillae, all being postanal in position with occasional variations in which the first lateral papilla may be on a level with or somewhat anterior to the anus; a third group of 2 pairs of lateral and 1 pair of ventral papillae in the region of the tip of the tail. Spicules, variable in size, minimum length 0.54 mm. in young forms and maximum length 2.4 mm. in adult forms; provided with sheaths. Spicules terminate in a rather prominent rounded enlargement (fig. 17). The sucker is circular, variable in size, measuring from 0.2 to 0.25 mm. in adult specimens; considerably smaller in immature forms. The length of the tail, which ends in a slender tip in adult forms, is from 0.5 to 0.7 mm.

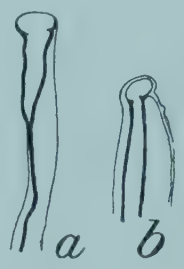

FIG. 17.—Tip of spicule of *Ascaridia lineata* viewed from different aspects (original)

FEMALE.—The vulva lies approximately at the middle of the body. Eggs thick-shelled, elliptical in shape, 80 μ long by 50 μ wide. Anus from 1.1 to 1.3 mm. from tip to tail in adult forms and shorter in immature forms. Tail ends in a slender tip (fig. 18).

HOSTS.—*Gallus domesticus, Anas boschas domesticus,* and *Anser domesticus.*

LOCALITIES.—Europe, North and South America, Asia, and Africa.

THE GENUS ASCARIDIA DUJARDIN, 1845

Railliet and Henry (*11*) define the genus Ascaridia as follows: Mouth with three lips; esophagus without a bulb, often with lateral membranes. Caudal alae of male feebly developed. Spicules equal or subequal, without an accessory piece. Preanal sucker rounded, with chitinous ring; papillae relatively large. Vulva toward the middle papillae of the body; uteri opposed; eggs thick-shelled.

In a paper on suckered roundworms, Lane (*5*) amends the diagnosis of the genus Ascaridia as given by Railliet and Henry, by adding the following generic characters: 10 pairs of caudal papillae in the male, and similar spicules. In a later paper Lane (*6*) discusses the generic diagnosis of Ascaridia in greater detail, insisting on the view that species having more than 10 pairs of papillae that are now assigned to the genus Ascaridia should be placed in another genus, but he makes no further reference to similarity in size of spicules. According to Travassos (*16*), the male of *Ascaridia columbae* has 14 pairs of papillae; that of *A. truncata,* the type species of the genus, 15 pairs, and that of *A. magalhaesi* 12 pairs. In addition

to the forms just mentioned, there are several species of Ascaridia that have more than 10 pairs of papillae in the male. So far as concerns *A. truncata* of Travassos, Lane considers it congeneric with *Gireterakis girardi*, a new genus and new species having a bulbed oesophagus, described by himself, and he therefore concludes that *A. truncata* of Travassos is not

FIG. 18.—Tail of female of *Ascaridia lineata* (original)

identical with *Fusaria truncata* of Zeder, the type species of the genus Ascaridia. Lane has assumed that the species described by Travassos as *A. truncata* possesses a bulbous oesophagus and therefore does not belong to the genus Ascaridia. In the writer's opinion this assumption is unwarranted. In the absence of definite information to the contrary it seems fairer to assume that the species designated by Travassos as *A. truncata* has a simple oesophagus characteristic of the genus Ascaridia, a fact which is clearly implied in the generic diagnosis of Ascaridia as given by Travassos. Lane's additional assumption that *A. truncata* of Travassos included more than one form must be left open to question in the absence of definite information on this point. The point to be considered in this connection is that *A. truncata* of Travassos is a parasite of birds, whereas *Gireterakis girardi* is a mammalian parasite, the difference in host relationship of the two forms favoring the probability of their being different genera.

One point on which Lane and Travassos agree, however, is the importance of the structure of the oesophagus in the classification of suckered roundworms. Lane (*6*), referring to the members of the genus Ascaridia, states that they will probably be subdivided in the future into several genera and will come to occupy the status of a subfamily at least, and that the genera Heterakis, Ganguleterakis, Gireterakis, and related forms having a bulbed oesophagus will constitute another subfamily. Future investigation may perhaps justify Lane's position, but there appears to be no need at the present time to limit the definition of the genus Ascaridia beyond what is given by Railliet and Henry (*11*).

SYSTEMATIC POSITION OF ASCARIDIA LINEATA

In common with other species of the genus, *Ascaridia lineata* is generally referred to the family Heterakidae, which is included in the superfamily Ascaroidea. According to Travassos (*17*), whose conception of the nematode superfamilies is to some extent at variance with that of Railliet and others, the genus Ascaridia belongs to the family Ascaridae, and not to the family Heterakidae, which he limits to forms having a bulbous oesophagus. Travassos furthermore assigns the family Heterakidae to the superfamily Oxyuroidea, thus placing the genus Ascaridia in a different superfamily, namely, Ascaroidea. This conception of the affinities of the genus Ascaridia differs radically from that of Railliet and Henry (*11*), who place Ascaridia with other suckered roundworms in the family Heterakidae. The genus Ascaridia has undoubted affinities with certain Ascaridae so far as concerns the structure of the oesophagus and the presence of dentigerous ridges on the lips, but so far as concerns the presence of a preanal sucker and a bursalike tail in the male, it is closely related to the Heterakidae.

The zoological position of the genus Ascaridia is a question that can not be finally settled until careful morphological comparisons are made not only of the adult forms, but also of the larvae at various stages of development, with those of corresponding stages of genera of Ascaridae on the one hand and with genera of Heterakidae on the other.

SUMMARY AND CONCLUSIONS

The principal facts presented in the foregoing pages may be summarized as follows:

Ascaridia lineata is the common intestinal roundworm of chickens in the United States, the occurrence of *A. perspicillum* in this country not having as yet been established on the basis of morphological comparisons of Ascaridia occurring in American chickens with the different forms that have been described as *A. perspicillum.*

So far as can be judged from the various illustrations of *Ascaridia perspicillum,* this species is either highly variable as regards the arrangement and location of the papillae in the tail of the male, or else several different species have been confused under one name.

Ascaridia lineata exhibits considerable individual variations, these being correlated with size and therefore with age of the different specimens. Of special interest in this connection is the variation in the length of the spicules, organs to which considerable specific importance is usually assigned by helminthologists. Of perhaps equal importance is the variation in the size of the sucker, which has a chitinized rim.

Ascaridia lineata is recorded for the first time as a parasite of the goose, on the basis of specimens from Indo-China.

In view of the apparent absence of *Ascaridia perspicillum* from American chickens, various facts published in the United States concerning the life history, pathology, and physiology of *A. perspicillum* as well as facts pertaining to medicinal treatment in all probability refer to *Ascaridia lineata.*

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SOME LABORATORY METHODS FOR PARASITOLOGICAL INVESTIGATIONS [1]

By MAURICE C. HALL and ELOISE B. CRAM, *Zoological Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The post-mortem examination of the viscera of animals for parasites constitutes a large part of the work of a laboratory of parasitology, and the efficiency of the work depends largely on the effectiveness of the methods used in such examinations. Owing to the general prevalence of parasites in the digestive tract, the examination of this tract is of special importance.

The method of examining the digestive tract which is most widely employed in laboratories is substantially as follows: The esophagus is removed, slit with the indispensable enterotome, and examined by reflected light for evidence of parasites superficially visible and by transmitted light for parasites embedded in the tissue and not superficially visible. The stomach is opened into a jar of water or physiologic saline solution, the contents washed out, the stomach itself examined internally, externally, and if possible by transmitted light for parasites present, and the stomach contents repeatedly sedimented and washed until the supernatant water or saline solution is clear, the sedimented contents then being examined a bit at a time after being poured into wide, shallow glass dishes. The small intestine or its component portions, the cecum, and the large intestine or its component portions are examined in the same manner as the stomach.

This method has long been in use and is, as a rule, a fairly satisfactory procedure. It is best suited for examining the digestive tract of such animals as dogs. In the case of a larger animal, such as a horse, cow, or sheep, the contents of the stomach and large intestine are so bulky that this method of examining is unsatisfactory, and as a rule the examination is confined to the region of the mucosa and the contents near the mucosa, the interior of the mass of ingesta or fecal material receiving little or no attention in many cases. This was the method employed by Looss [2] in examining horses for parasites. In some cases the contents of the large intestine (cecum and colon) of the horse are examined by pressing the material into balls and picking these apart by hand. This was the method employed by Hall, Wilson, and Wigdor [3] and they used the same method for examining the manure of horses for worms passed after anthelmintic treatment.

THE USE OF SCREENS

It has occurred to the writers that the use of the graduated set of screens which they find indispensable in examining feces for parasite eggs and for worms passed after anthelmintic treatment in the case of dogs and similar small animals, might be of value in post-mortem examination as well. Various other writers, such as Stiles, Cobb, Telemann, Bass, and Garrison, had advocated the use of a screen or sieve of metal, gauze, or bolting cloth in the examination of feces for parasite eggs; but the advantage of a set of metal screens of graduated sizes, set in a rack in the order of size with the largest-mesh screen at the top and the smallest-mesh screen at the bottom, appears to have been emphasized first by Hall.[4] This set of screens was also used in examining feces for worms passed after anthelmintic treatment in critical testing of anthelmintics by Hall and Foster.[5] The usefulness of assorted screens for the separation of mixtures of objects of various sizes is generally recognized in industrial procedures. Feces and gastro-intestinal contents are mixtures of this sort.

[1] Received for publication June 30, 1924; issued June, 1925.

[2] LOOSS, A. THE SCLEROSTOMIDAE OF HORSES AND DONKEYS IN EGYPT. Rec. Egyptian Govt. School Med. 1901: 25–138, illus. 1901.

[3] HALL, M. C., WILSON, R. H., and WIGDOR, M. THE ANTHELMINTIC TREATMENT OF EQUINE INTESTINAL STRONGYLIDOSIS. Jour. Amer. Vet. Med. Assoc. (n. s. 7) 54: 47–55. 1918.

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[5] HALL, M. C., and FOSTER, W. D. EFFICACY OF SOME ANTHELMINTICS. Jour. Agr. Research 12: 397, 447, illus. 1918.

In their experiments, the writers have substituted the use of screens for the sedimentation and washing method in examining the digestive tract in all cases where such a substitution saved time. The screens used were the same ones used by Hall and by Hall and Foster. These screens were made by taking round tin pans with a bottom diameter of about 6.5 inches and a slightly greater top diameter, with sides 2 inches high, and with a projecting flange rim at the top, cutting out most of the bottom of the pan, but leaving a small flange projecting inward, and soldering brass screening of assorted sizes to the bottom flanges of the various pans. Some of the pans were enameled and some shellacked to prevent rusting. The enamel and shellac have never been renewed since the first coat, and these screens—very cheap affairs to begin with—are still serviceable after about 14 years of constant use. The expense of screens is therefore a very small matter, and even poorly equipped laboratories can easily afford them.

A much better set of screens for the purpose has been described by Hall.[6] These screens (fig. 1) are of copper and are about 7⅞ inches (20 cm.) square in inside dimensions. They are made of two copper strips, swaged together at two diagonal corners, the top half-inch of the metal being bent over and doubled back against the side on two opposite sides to form a reinforced flange ¼-inch wide which carries the screen in a rack. The copper sides are 2 inches high. On the bottom of the screens the metal is bent in to form a flange ⅜ inch wide for the attachment of the brass screening, which is soldered to this flange. The screens described had mesh apertures of 6, 8, 10, 14, 16, 20, 60, 100, and 120 to the inch, the latter being about the finest screen that will permit of the passage of the eggs of practically any worm parasite. Each sieve has a number stamped in the front side to show its number of mesh apertures to the inch. A solid-bottomed copper pan, of the same shape and dimensions as the screens, completes the set. A rack with grooves in its solid board sides or with transverse pieces on the sides and back of a skeleton construction of upright pieces is provided, and the screens are supported by the grooves or transverse pieces when they are set up in the rack. The number of screens used in any operation will vary with the nature of the material examined and the judgment of the operator.

The material which the writers have examined post mortem by means of screens consisted mostly of the contents of digestive tracts of dogs killed at the end of experiments in critical testing of anthelmintics, and of digestive tracts of wolves, coyotes, lynxes, and one bear sent in by the field service of the Biological Survey of the Department of Agriculture in response to a request by the Zoological Division for this material. The viscera of the dogs were fresh. The viscera of the wild carnivores were sent from the field wrapped in borax or in cloth saturated in formaldehyde solution, some of them coming from as far as California, and were often a week or ten days old when received.

THE SCREENING METHOD

The method of screening the contents of practically all portions of the digestive tract, including the stomach, small intestine, cecum, and large intestine, was first tested, each of these portions being opened separately into a jar of water and each screened separately. For this purpose the three screens having 6, 12, and 24 mesh apertures to the inch were generally used. The finest of these screens shows some little variation in its mesh apertures and may have in places 21 or 22 mesh apertures to the inch. It is not so fine as could be desired for the final screen, and the 60-mesh screen was sometimes used as a final screen. One with a mesh aperture of perhaps 40 to the inch should probably be added to the set. The number of screens and the mesh sizes to use are matters of individual judgment. The screens should be used in a rack for various reasons, one reason being that this results in cutting down the water pressure on the last screen and diminishing the likelihood of washing worms through that screen by high-water pressure.

The advantages of this method over the sedimentation and washing method are very evident. As soon as the contents of a jar are poured on the screen, a hose which is attached to a water tap having a foot-pedal control for the flow is used to wash the material on the top screen until it is clean and comminuted as much as possible. The pressure of the water and the amount of washing are matters of individual judgment. The water runs

[6] HALL, M. C. APPARATUS FOR USE IN EXAMINING FECES FOR EVIDENCES OF PARASITISM. Jour. Lab. and Clin. Med. 2: 347–353, illus. 1917.

through to the lower screens and washes the contents which pass through from the top screen. Most of the unpleasant odor of the ingesta and fecal material (and the odor of viscera packed in borax for 10 days is decidedly unpleasant) is associated with the fine material which passes through the last screen, and this is promptly washed down the sink in which the rack is set. The less unpleasant material, with whatever parasites may be present, remains on the screens and is transferred to shallow glass dishes either by washing or by immersion in water in the dishes and tipping to remove all or part of the contents at a time. In either case the screens are placed in water in the dishes and examined for parasites that may be held on them or in their meshes.

In most cases this method of examination shows the following advantages over the sedimentation and washing method: There is a great saving of time, as washing through the screens is a much quicker process than sedimenting and washing repeatedly to remove the supernatant discolored fluid and flocculent material. The parasites are washed out of the ingesta, making their recognition and collection easier and more certain. The malodorous material is removed much more promptly and the entire process of examination is much pleasanter.

FIG. 1.—A set of copper screens, some on skeleton rack with transverse pieces at left, some in solid board rack with grooves at right, one on edge at bottom of left-hand rack, and one, seen partly from above, on the table in the center; solid copper pan on edge at right. (From Hall, 1917.)

The disadvantages of this method of screening are mostly theoretical and generally follow errors of judgment. In the first place, it was found that in practice time could not always be saved by substituting the screening method for the sedimentation method. These cases were usually those in which washing itself was unnecessary. Such cases include the examination of the practically empty stomach of a fasted dog or the almost empty cecum of individual carnivores of any sort. In these cases the organs in question could be slit open into a jar, the material allowed to settle for a few minutes, the supernatant fluid decanted, and the small sediment examined immediately, or after one washing, in a glass dish. This is a quicker procedure than screening and examining the screens, and there is little in the way of ingesta or fecal material to conceal parasites or afford unpleasant odors.

Another objection is on the score of possible injury to parasites by screening and washing. For the practical purposes of routine examination for and collection of parasites, this objection is mostly theoretical, although in special cases it would be sound. In general, worm parasites are fairly resistant structures as met with in the field of human and veterinary medicine. Such worms as the ascarids are fairly tough structures, and *Ascaris lumbricoides* has been kept alive in Kronecker's solution for 26 days, which shows that it is not readily damaged by being removed from its host and placed in an alien environment. It is true that there are some very fragile worms that are very susceptible to mechanical injury and the destructive action of osmosis in such fluids as tap water, but worm parasites of the digestive tract are rarely of this sort. It is also true that the easily lost hooks of some tapeworms may be lost by screening.

Where worms are being collected for very careful studies of their morphology a more refined technic is desirable; but for practical routine purposes the writers find the screening method superior to the sedimentation and washing process, provided one uses good judgment, and believe that the contents of the small and large intestines may be screened to advantage in almost all cases and that screening of the contents of the stomach and cecum should be carried out when of sufficient quantity to warrant it or omitted if of such small quantity that little or no washing is necessary. The screening method would be of value for the examination of swine viscera and to some extent for the examination of the viscera of such animals as sheep, cattle, and horses.

In connection with the subject of laboratory technic it is also noted that it is advisable to slit the larger air passages of the lungs and then wash and squeeze the lung in a dish of water or saline solution in order to detect worms which may be overlooked in slitting and examining without this washing. The urinary bladder, gall bladder, and similar structures also should be slit in a dish of water or saline solution and examined in the same way and for the same reason.

CONCLUSIONS

A set of metal screens of assorted mesh apertures with a suitable rack to hold them is a very valuable and almost indispensable part of the equipment of a parasitological laboratory. The screens are of service in examining feces for parasite eggs and for worms passed after anthelmintic treatment and in examining the contents of the digestive tract post-mortem. If used with judgment in post-mortem examinations they save time, make the detection and collection of parasites easier and more certain, and make the work less unpleasant by removing the malodorous portions of ingesta and fecal material more rapidly than the sedimentation and washing method does. There appears to be little damage done to parasites, as a rule, by the use of screens and washing. The lungs, urinary bladder, gall bladder, etc., should be slit open in a dish of water or saline solution and examined in this way for parasites present.

SOME LEPIDOPTEROUS LARVAE RESEMBLING THE EUROPEAN CORN BORER[1]

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INTRODUCTION

The European corn borer (*Pyrausta nubilalis* Hübner) is unquestionably the most important potential insect enemy of the corn plant in the United States and Canada. Inasmuch as there are other lepidopterous larvae attacking corn which may be readily confused with the European corn borer, it seems desirable to give an account of these larvae, to point out the structural characters wherein they differ from one another, and briefly to discuss their habits and seasonal histories in order further to facilitate the identification of the European corn borer in the field, as has been done by Heinrich (*13*)[3] for the pink bollworm and lepidopterous larvae likely to be confused with it.

The larvae of two native species, *Pyrausta ainsliei* Heinrich and *P. penitalis* Grote, which also inhabit corn at certain seasons of the year, so closely resemble the larvae of *Pyrausta nubilalis* in color, size, and structure that their identification without final resort to the microscope will always remain uncertain. Immature larval stages of these three forms are especially difficult to separate. Other plants than corn attacked by the European corn borer are hosts of lepidopterous borers and surface feeders, some of which, although entirely unrelated to one another, resemble more or less closely the larvae of *P. nubilalis*. In order to draw a line, however, as to the forms to be included in this category, special consideration is given only to those larvae inhabiting the preferred food plants of the European corn borer. Field observations in connection with the species discussed were conducted in the Massachusetts area infested by the corn borer, unless otherwise indicated.

It was necessary in this work to make distinctions between food plants and shelter plants, as Ainslie and Cartwright (*1, p. 837*) also found necessary in their work on the biology of *Pyrausta ainsliei* in Tennessee. Generally, the naming of a particular plant as a host plant implies that the insect feeds upon it and derives its livelihood therefrom. This is not strictly true, however, for some lepidopterous larvae boring in weeds and other plants migrate at certain seasons of the year, particularly in the autumn, to other apparently more suitable plants in which they find desirable quarters for the winter (*2*). Usually the larvae possessing this migratory habit—those observed—are full grown in the fall and consequently require no further food in the spring for the continuance of their development. They have lived at the expense of small marrowed or pithy stems. When completely tunneled out, these stems are obviously frail and when subjected to the rigors of winter in the north are readily broken over, frequently exposing the burrows of the larvae within. Thus the larvae are feebly protected unless they migrate to more favorable situations or mine into the underground stems of the plants they infest, as do Gnorimoschema and Pterophorus in *Solidago sempervirens* L. Plants which afford only wintering or pupation quarters for an insect should be termed shelter plants. The term "host plant" is relative.

Eleven species are considered in the present paper. Descriptive matter in the text has been reduced to a minimum; the important differentiating structural characters, however, are pointed out and further emphasized by figures.

[1] Received for publication Apr. 22, 1924; issued June, 1925. This study was projected by D. J. Caffrey, in charge of the European corn borer investigations, as an aid for field men. The writer materially benefited from his enthusiasm, suggestive criticisms, and general supervision of the work, and desires hereby to express his appreciation and thanks. It should also be stated that Carl Heinrich, of the Bureau of Entomology, read the manuscript, pointed out omissions, and suggested several important changes both in the text and in the figures.

[2] Resigned Jan. 12, 1925.

[3] Reference is made by number (italic) to "Literature cited," p. 792.

FAMILY PYRALIDAE

Subfamily Pyraustinae

PYRAUSTA NUBILALIS HÜBNER

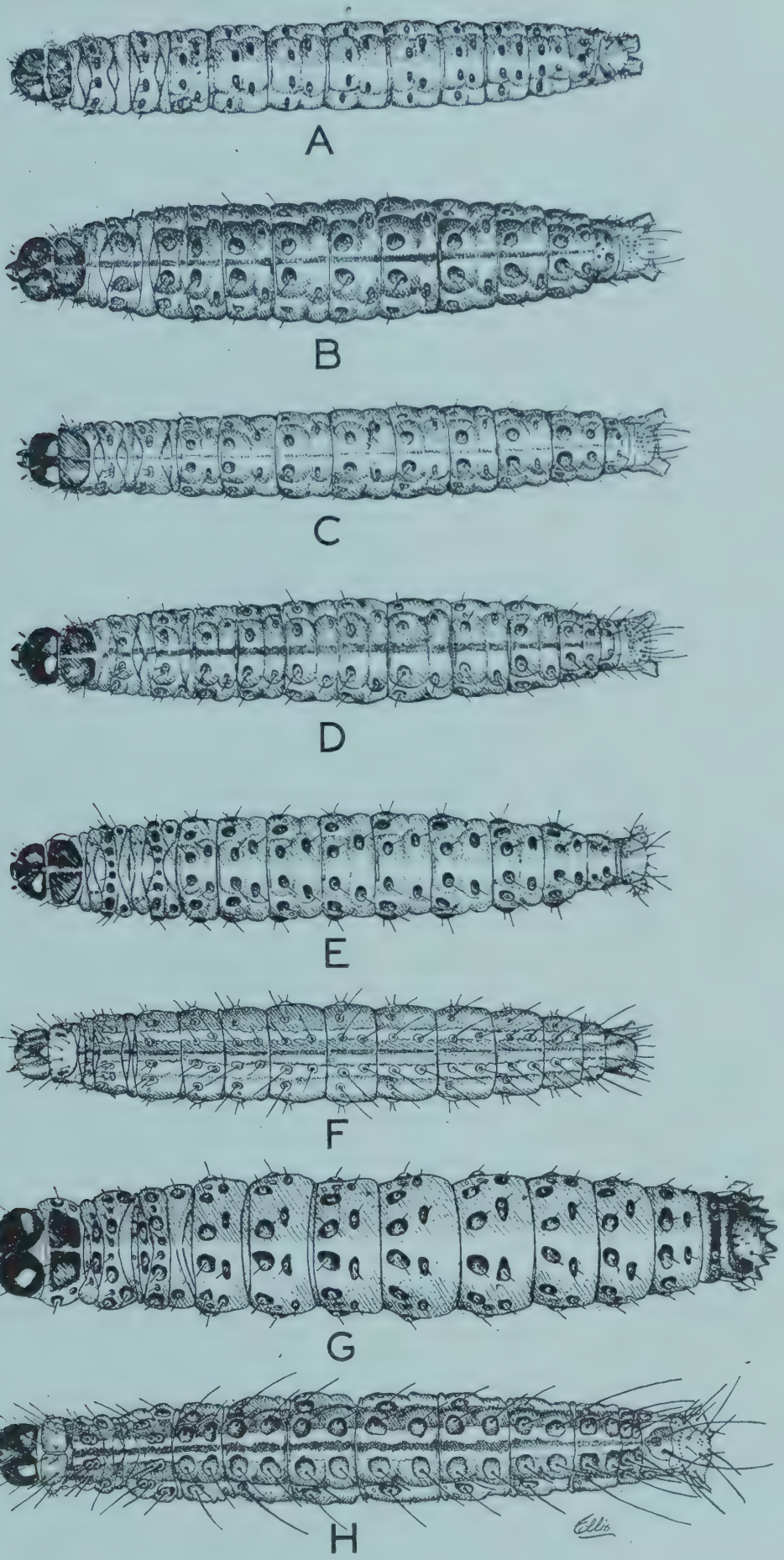
Pyralis nubilalis Hübn., 1796, Eur. Schmett., Sechste Horde, 25, 14, Pyr., pl. 14, fig. 94.
Pyralis silacealis Hübn., 1796, Eur. Schmett., Sechste Horde, 25, 15, Pyr., pl. 18, fig. 116.
Pyralis glabralis Haw., 1811, Lepidop. Brit., p. 380.
Botys lupulinalis Clerck, Guenée, 1854, Delt. et Pyr. (Bdv. et Guen., Hist. Nat. Ins. Lépidop.) 8: 331.
Botys zealis Guen., 1854, Delt. et Pyr. (Bdv. et Guen., Hist. Nat. Ins. Lépidop.) 8: 332.
Botys lupulina Heinemann (nec Clerck), 1865, Die Schmett. Deutsch. und der Schweiz, 1, 2, 70.
Hapalia kasmirica Moore, 1888, New Ind. Lepidop. Ins., p. 222, pl. 7.
Hapalia lupulina Butler (nec Clerck), 1889, Illus. Typ. Spec. Lepidop. Het. Coll. Brit. Mus. 7: 19.
Pyrausta nubilalis Hübn., 1901, Staud. and Rebel, Cat. Lepidop. Aufl. 3, 2: 65, No. 1218.

For complete information on the status, habits, life history, etc., see paper by Vinal and Caffrey (*21*).

The European corn borer (*Pyrausta nubilalis* Hübn.) was described by Hübner in 1796 as *Pyralis nubilalis*. The description consisted of a careful drawing of the male and a descriptive note added to the text of "Sechste Horde." Hübner also described the female as *silacealis* in the same manner with a figure and a note, apparently not recognizing, owing to the complete difference in coloration of the two sexes, that this species was the female of his *nubilalis*. It seems that Clerck (*3, Sect. I, pl. 9, fig. 4*) in 1759 attempted to describe a species under the name *Phalaena lupulina*, but executed so poor a figure that the majority of workers have disregarded it. Guenée, although admitting that Clerck's figure is poor, believed that *lupulina* should take precedence over Hübner's *nubilalis*, since he was convinced that they were identical. He therefore assigned the name *Botys lupulinalis* to the species, giving Clerck credit for the authorship, and several succeeding writers have used the name *lupulina* of Clerck for Hübner's species. A study of Clerck's figure by later workers, however, has convinced them that Clerck's *lupulina* is not identical with Hübner's *nubilalis*. Since then the species has been described under a variety of names, but all recent catalogues on Lepidoptera maintain the name *nubilalis* Hübner. On account of priority, the generic name Botys erected by Latreille in 1802 has been supplanted by Schrank's Pyrausta of 1801.

The full-grown larva of the European corn borer (description, *11, p. 174*) (pl. 1, D) averages 0.81 of an inch in length, or 19.95 mm. (*20, p. 27*). The integument on the dorsal side of the larva is heavily granulated, the granulations extending to the pleura of the body segments. Laterad and ventrad the integument is a dirty white. The skin granules carry the pigmentation which varies from a pink, slate gray, or "smoky-fuscous" (*11*) to a light brown. Since each color may predominate over the other, various color combinations are present in living larvae which are obviously difficult to describe. The skin granules are most dense on the dorso-median plane and take the form of a distinct stripe which is more or less interrupted where the integument folds in on the dorsal side of the larva to delimit the body segments; notwithstanding this interruption, the stripe is plainly visible to the unaided eye as a dark, pigmented, longitudinal band. On the larva of *Pyrausta ainsliei*, a close relative of the larva of the European corn borer and almost identical with it structurally, this stripe or band is very insignificant (pl. 1, C), narrow, and difficult to establish as present. This difference has been found to be a safe field character for the separation of full-grown living larvae. This character can not be depended upon for use in separating material preserved in alcohol, as it is a well-known fact that even with the utmost care in preservation pigmented areas fade.

The abdominal segments bear on the integument of the dorsum or in its creases and folds small rounded clear areas, some of which appear fused with each other, forming thereby somewhat irregular moniliform clear spaces. Miss Mosher endeavored to establish the constancy of these areas on the abdominal segments (*18, p. 265*) and used them in separating *nubilalis* from *ainsliei*. Heinrich has pointed out (*11, p. 176*) that this character is elusive and unreliable. It is possible to find in a small series of selected larvae some constancy as to the number and placement of these clear areas, but in a large series of larvae the character is at once seen to be inadequate and unreliable. As to the morphology of these areas Heinrich believes by analogy that they afford attachment for certain muscles and homologizes them with similar weakly chitinized areas in certain Phycitinae—notably Dioryctria and Pinipestis by way of example (*11, p. 174, footnote*). Microscopic sections through these areas



DORSAL VIEW OF LARVAE

A.—Bidens borer, *Epiblema scudderiana*
B.—Nelumbo borer, *Pyrausta penitalis*
C.—Polygonum borer, *P. ainsliei*
D.—European corn borer, *P. nubilalis*
E.—Parsnip webworm, *Depressaria heracliana*
F.—Greenhouse leaf-tyer, *Phlyctaenia rubigalis*
G.—Spindleworm, *Achatodes zeae*
H.—Celery stalkworm, *Nomophila noctuella*

fail to reveal that they afford attachment for body muscles, cutaneous or others. It is true that they are feebly chitinized, thinner in fact than other portions of the integument, but this is apparently due in large measure to the absence of the characteristic skin granules present elsewhere on the dorsum.

Heinrich uses the anterior epicranial setal group (*11*) and puncture to separate *nubilalis* from *ainsliei*. This unquestionably is a good character, and is, as a matter of fact, the only reliable character that can be used to separate the two species in all larval stages (see *P. ainsliei* below) (pl. 2, I and J).

PYRAUSTA AINSLIEI HEINRICH

Pyrausta ainsliei Heinr. 1919, Jour. Agr. Research 18:175, pls. 7–10.
Pyrausta penitalis auct. (nec Grote).

After a careful comparison of the genitalia of the specimens of *Pyrausta penitalis* Grote in the National Museum and after a further examination of Grote's types of the same species in the American Museum of Natural History in New York, Heinrich was convinced that two species were involved and confused with each other under the specific name *penitalis*. Accordingly, he separated this species and described it as *ainsliei* from specimens furnished him by George G. Ainslie of the Bureau of Entomology, Knoxville, Tenn.

In 1920 Flint and Malloch treated a lepidopterous larva occurring in smartweed as Lederer's *obumbratalis* (*8, p. 303–304*). Heinrich (*12, p. 57*) established the identity of this species as *ainsliei*, admitting the possible synonymy but calling attention to the impossibility of fixing the actual identity of *obumbratalis* at the present time. To avoid confusion and possibly misapplication of a name, it is advisable at present to use *ainsliei* rather than *obumbratalis* as the designation for our smartweed borer.

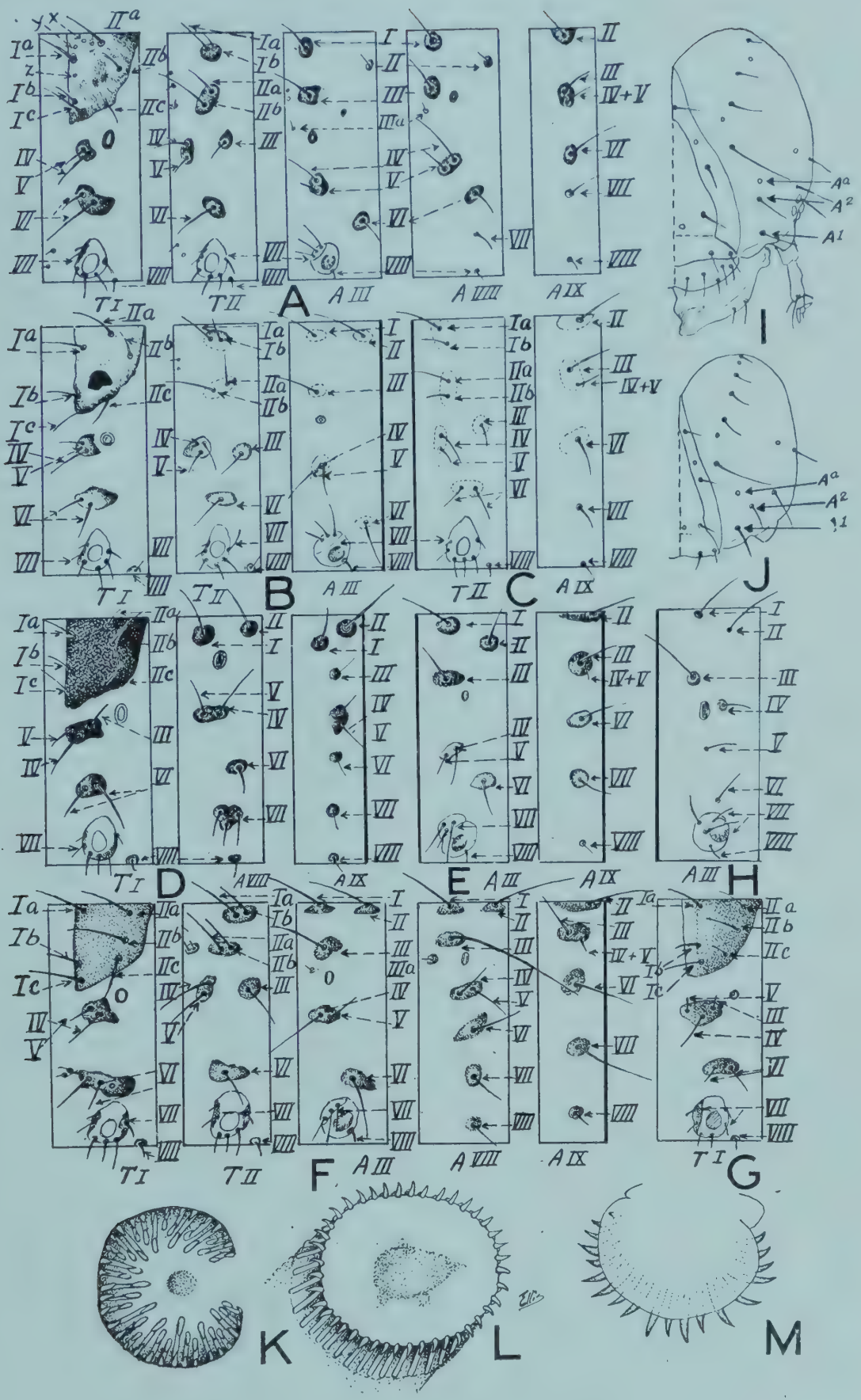
The larvae of *Pyrausta ainsliei* have been found in all stages of development in eastern Massachusetts in the following plants: *Polygonum pennsylvanicum* L. var. *laevigatum* Fernald, *P. lapathifolium* L., *P. persicaria* L. (smartweeds); *Xanthium* sp. (cocklebur); *Ambrosia artemisiaefolia* L. (ragweed); *Eupatorium* sp.? (Joe-pye weed); *Apocynum androsaemifolium* L. (spreading dogbane); *Typha latifolia* L. (cat-tail); *Chenopodium album* L. (lamb's-quarters). Adults have been bred through from second instar larvae from all these plants with the exception of Apocynum and Chenopodium. In these two cases very young larvae were taken from these plants in the field but failed to live in confinement. By subsequent visits to plants infested in the field, however, the writer was convinced that the species was developing therein. It should be stated in this connection that instances of larvae developing in plants other than *Polygonum pennsylvanicum* L., and *P. lapathifolium* L., were infrequent; so that it became apparent that the species greatly prefers the smartweed group of plants in which to breed. In the late autumn larvae have been taken from the following shelter plants: *Rubus* sp.?; *Sambucus canadensis* L. *Zea mays* (corn), *Solidago canadensis* L., *Echinochloa crusgalli* (L.) Beau. (barnyard grass), *Bidens frondosa* L. (beggar-ticks); *Typha latifolia* L., *Apocynum androsaemifolium* L. In November Vickery sent a lot of cotton plants collected at Wilmington, N. C., February 11, 1921, to the Arlington, Mass., laboratory, from which many larvae of *Pyrausta ainsliei* were cut. Cotton is probably a shelter plant.

Ainslie and Cartwright published some results obtained on the biology of *ainsliei* in which they list (*1, p. 838*) the following as reported natural food plants: *Polygonum pennsylvanicum* (var. not given), *P. lapathifolium, P. persicaria, P. hydropiperoides.* They also reared the larvae in confinement

EXPLANATORY LEGEND FOR PLATE 2

LARVAL DETAILS

A.—Setal map of *Pyrausta nubilalis*, prothoracic, mesothoracic, and third, eighth, and ninth abdominal segments of larva. (After Heinrich)
B.—Setal map of *Phlyctaenia rubigalis*, prothoracic, mesothoracic, and third abdominal segments
C.—Setal map of *Diatraea zeacolella*, mesothoracic and ninth abdominal segments. (*14, pl. 4*)
D.—Setal map of *Depressaria heracliana*, prothoracic and eighth and ninth abdominal segments
E.—Setal map of *Loxostege similalis*, third and ninth abdominal segments
F.—Setal map of *Nomophila noctuella*, prothoracic, mesothoracic, and third, eighth, and ninth abdominal segments
G.—*Epiblema scudderiana:* Setal map of prothorax
H.—*Heliothis obsoleta:* Setal map of third abdominal segment
I.—*Pyrausta nubilalis:* Cephalic view, left half of head
J.—*P. ainsliei:* Cephalic view, left half of head capsule
K.—*P. nubilalis:* Crochets on plantum of pseudopodium
L.—*Epiblema scudderiana:* Crochets on plantum of pseudopodium
M.—*Heliothis obsoleta:* Crochets on plantum of pseudopodium. (After Heinrich)



(For explanatory legend see p. 780)

on the leaves of *Rumex crispus* (curled dock) and *Fagopyrum fagopyrum* (buckwheat).[4] These workers have found the larvae in the following plants, which they assume to be purely shelter plants: *Zea mays* (corn), *Ambrosia trifida* and *A. artemisiaefolia* (ragweeds) *Xanthium communis*, cocklebur) *Solidago* spp. (goldenrod), *Aster* spp. (aster), *Phleum pratense* (timothy), *Typha latifolia* (cat-tail), *Bidens bipinnata* and *B. frondosa* (beggarticks), *Brassica arvensis* (wild mustard) (recorded by Felt), *Eupatorium* sp.? (Joe-pye weed) (recorded by Chittenden). Since both *nubilalis* and *ainsliei* possess this migratory habit, the former perhaps to a less degree, it will not be surprising if the larvae are taken in many plants and in other situations affording suitable quarters in which to pass the winter. Flint and Malloch (*8, p. 293*) published a substantial list of plants in which the larvae of *ainsliei* have been taken in the fall.

It is amazing that a species so abundant and so widely distributed as *ainsliei* should have remained undiscovered until so recent a time. It occurs abundantly wherever it has been reported as present, from Quebec, south through New England, New York, New Jersey, Pennsylvania, Maryland, Virginia, the Carolinas, and Tennessee, to Florida, west through Mississippi to Louisiana, and north through Kansas and Iowa to Michigan. It doubtless has a range even greater than we know of at present.

In Massachusetts the insect has a single brood (very often a partial second), in New York apparently one generation (Bartley and Hofer[5]); in Northern Ohio apparently two generations (Poos[5]); in Iowa two generations (*20*); in Tennessee two generations (*1*); and in Mississippi three generations (Allen[5]). In New England the moths appeared in 1921 the first week in June, emergence continuing through July 10, on which date the last pupa was observed in the field. Larvae were full grown by September 1, although many immature were also present in the field. On September 12, 1921, the writer first observed the larvae in corn for the season and from this date until October 5 their numbers appeared to increase constantly in corn plantations and in other places of shelter. In confinement, pupae remained as such for an average period of 15.3 days.[6] Egg masses hatched on the average in 6.5 days.

Small holes in the stalks of Polygonum and other plants with frass extruding and suspended somewhat beneath the openings indicate where the borers are at work in the plant. The tissue about the opening is usually slightly sunken and discolored. Since a large number of borers can be found at work in a single plant it is common to find the Polygonums on dumps and at wayside places in a state of utter collapse. On opening such infested plants one finds in place of the customary pith and fleshy medulla, a mass of castings, larvae, and larval exuviae. Prior to pupation, the larvae cut exits in the stalk for the convenience of the moths, covering these openings with tympana of silk. The larvae rarely mine through the septa of the nodes of Polygonum but confine their feeding almost exclusively to the internodes. The larvae are much less active than the larvae of *Pyrausta nubilalis;* in fact, they are comparatively sluggish. Corn borers, on the slightest disturbance or provocation, move out of their quarters; *ainsliei* larvae remain to be prodded out or summarily removed by hand.

Observations made by the writer in New England on the developing larvae do not completely coincide with those made by Ainslie and Cartwright in Tennessee (*1, p. 840*). The young larvae, for the most part, do not at once enter the stems of Polygonum, but invariably on emergence from the eggs they freely pit the midrib and feed on the epidermis of the under surface of the leaves which hold the egg masses. During the time the larvae are thus engaged the chitin of the mandibles fully hardens and the head capsule assumes its normal pigmentation. It is true that immediately following their escape from the eggs the young larvae remain assembled in gregarious fashion, but in the course of a day they disperse,

[4] In this connection Ainslie and Cartwright also state (*1, p. 838*): "Leaves of all common weeds and plants were offered to the larvae, but in every case except the two mentioned above they were either refused or only slightly gnawed." This general statement implies that all common weeds and plants from various sections of the country were offered to the larvae. These investigators probably mean that the most common weeds and plants in the vicinity of Knoxville, Tenn., were utilized in experimentation.

[5] Unpublished.

[6] Wm. B. Turner, of the Sacramento Entomological Laboratory of Cereal and Forage Insect Investigations, was connected with the European Corn Borer Laboratory and directly associated with the work in 1920 and 1921. He secured the pupation records, devised cages, and contributed other important data to our knowledge of *P. ainslici*. Mr. Turner died June 12, 1924.

entering the stems of the plant near where the eggs were deposited. As a result of larval feeding these small stems wilt. The larvae, therefore, leave these, migrating elsewhere to another section of the plant, and they may do so several times in the course of their development. Other pyraustine larvae have been observed to emerge from the stem in which they are feeding to pass through their first larval molt. This habit of migrating apparently works hardship on the species, for doubtless it accounts for the relatively high parasitism in larvae of *Pyrausta ainsliei*.

Egg masses were found to contain anywhere from 2 to 35 eggs. Ressler (*20, p. 278*) records having secured 50 in a single cluster. An average female probably lays 300 to 600 eggs. It is possible to distinguish very young larvae and egg masses of *Pyrausta ainsliei* about to hatch from those of *P. nubilalis* in the same condition wholly on a head capsule comparison. The head capsule of *nubilalis* showing through the transparent corium of the egg when the latter is about to hatch is is always jet black; in *ainsliei* it is a light straw or pale tan color and only assumes its characteristic deep chestnut brown or black color 18 to 24 hours after emergence from the egg.

The following parasites have been reared from *Pyrausta ainsliei* larvae at the Arlington laboratory. This list has been furnished by Dettmar W. Jones: *Microbracon* n. sp.; *Panzeria penitalis* Coq.; *Itoplectis conquisitor* Say; *Bassus agilis* Cress.; *Glypta rufiscutellaris* Cress.; *Ephialtes aequalis* Prov.; *Exorista nigripalpis* Town.; *Rogas rileyi* Cress. *Microgaster epagoges* Gahan is probably a primary parasite of *ainsliei*. Ressler (*20, p. 280*) in Iowa records that he reared a braconid, belonging to the genus Aleoides, from this species. Ainslie and Cartwright reared a predator (*1, p. 844*) in *Callida decora* Fab. They suggest that doubtless larvae of *Chauliognathus pennsylvanicus* De Geer destroy some of the borers; "in two instances they have been found feeding upon the contents of the puparia [doubtless they mean pupae] in the stems."

The larvae are cylindrical, 18.5 mm., or 0.718 inch, in length when full grown (pl. 1, C). They do not vary in color so markedly as the larvae of *P. nubilalis*. The larvae are for the most part slate gray and plumbeous colored on the dorsum and a dirty white ventrad. The head capsule is usually a deep chestnut brown, yet specimens may be had with black head capsules. On hatching from the egg, however, the head capsules of the larvae are always pale.

From an examination of thousands of living specimens at the European Corn Borer Laboratory during 1920 and 1921 it has become evident that Carl Heinrich's (*11*) structural distinction between *Pyrausta ainsliei* and *P. nubilalis* larvae is the only positive one, so similar is the morphology of these larvae. It is a difficult character to establish with a hand lens, owing to the dark pigmentation of the head capsules. This fact, unfortunately, hampers its usefulness for field men. Nevertheless the distinction is always constant and can be used in every instar. Other characters have been tested and tables kept to establish their reliability, such as the shape of the anal plate, the disposition of the clear spaces on the integument, and the distance between certain tubercles on particular abdominal segments, but invariably these have had to be given up because of their inconstancy.

Heinrich's character bears on the arrangement of the setae and the position of the puncture in the anterior epicranial group (*11, p. 178*) (pl. 2, I, J).

Epicranial setae and puncture A^1, A^2, and A^a forming an obtuse angle with A^a postero-dorsad of A^2—*P. ainsliei*.

Epicranial setae and puncture A^1, A^2, and A^a lying in a straight line or with A^a somewhat postero-laterad of A^2, not postero-dorsad—*P. nubilalis*.

Owing to the fact that the head capsules of these larvae are somewhat globose in form it is probable that Heinrich in the latter instance, when he refers to A^1, A^2, and the puncture A^a as lying in a straight line, actually means that their position is along an arc conforming to the declivity of the head.

PYRAUSTA PENITALIS GROTE

Pyrausta penitalis Grote, 1876, Canad. Ent. 8:98; Dyar, 1902, List N. Amer. Lepidop, No. 4439; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer. No. 5129.
Pyrausta nelumbialis Smith, 1890, Ent. Amer. 6:89.

In 1876 Grote described *Pyrausta penitalis* (the Nelumbo borer) from moths reared by Snow, of Lawrence, Kans. In 1890 J. B. Smith described the same species under the appropriate name of *nelumbialis*. He reared moths from larvae taken in the buds, flowers, seed capsules, and leaf and flower stems of the Egyptian lotus, collected at Bordentown, N. J.

During 1920 and 1921 the larvae of *Pyrausta penitalis* (pl. 1, B) were received at the European Corn Borer

Laboratory in the following plants: Egyptian lotus (Weiss, Riverton, N. J.); *Nelumbo lutea* (Wild.) Pers. (Howell, South Bass Island, Ohio); *Polygonum lapathifolium* L. (Craft, Sandusky, Ohio); *Polygonum pennsylvanicum* L. (Howell, Middle Bass Island, Ohio); rhubarb (G. A. Runner, Sandusky, Ohio). The rhubarb contained a great many larvae from the second to the fifth instars which were reared to adult moths. The rhubarb was badly damaged, entirely unfit for marketing. Just as the larvae of *nubilalis* in rhubarb eject gelatinous and gumlike castings from their burrows, so the larvae of *penitalis* feeding in the stems of the same plant push out of their tunnels similar exudations. The discovery of the larvae in rhubarb by Runner is apparently the first record for this species of economic note. F. W. Poos, Sandusky, Ohio, has since established beyond question the fact that the rhubarb in this case was not a host but a shelter plant. A large number of infested Nelumbo blooms were placed near the rhubarb plants. As these wilted the larvae deserted them for the rhubarb. Furthermore, Poos has been unable to rear the larvae on rhubarb and has likewise failed to find additional evidence that this species attacks rhubarb in the open. Vickery found the larvae of *penitalis* frequently in corn at Bay, Ohio, on September 13, 1921. These larvae were full grown and apparently were using the corn, as pointed out for *ainsliei*, as a place of shelter.

The Nelumbo borer is known to occur from Maine south through New Jersey, Washington, D. C., Tennessee and Missouri to Texas, and north through Kansas, Illinois, and Ohio.

Although *Pyrausta penitalis* has been collected in New England, the writer was unable during 1920 and 1921 to locate an infestation of this insect and, consequently, it was not possible to observe it under field conditions. Larvae sent to the Arlington laboratory by various field men in Ohio emerged as moths in the insectary in 1921 over a period of 18 days, from June 9 to June 27. An average of 14.7 days was spent in the pupal stage. Offspring from these moths brought through on *Polygonum pennsylvanicum* L. var. *laevigatum* Fernald were full grown larvae September 10. Howell and Vickery in August, 1921, found larvae commonly in all stages of development in Polygonum in northern Ohio. Apparently there is but one generation in Massachusetts. Poos finds three generations at Sandusky, Ohio.

The larvae have much the same habits as *P. nubilalis* and *P. ainsliei* in that they mine out the pith columns and feed on the medulla of the plants they infest, extruding frass from their burrows. Usually they spend more time in the open after hatching from the eggs than either of the above mentioned species, feeding on the leaf and flower buds, and the tender foliage. In confinement they have been observed to web together immature foliage and to feed therein for a considerable time prior to entering the stems of the plant. The larvae also appear to possess a greater silk-spinning ability than either *nubilalis* or *ainsliei*. When half grown, or before, they enter the plant and form definite burrows. While the larvae possess no particular body modification for aquatic life they are capable of remaining in water for days. In lotus stems they have been submerged for a month without apparent injury. Larvae on the surface film of water attain the sides of the vessel in which they are confined by convulsing the abdominal segments from side to side. Observers have noted that the larvae of this species are not to be found in lotus plants in the autumn, but in hollow reeds or other places of shelter on shore (*2*). To remain in the old lotus or Nelumbo plants as they collapse and fall into the water in the autumn, remaining submerged over the winter, apparently would not make for the welfare of the species. It was noted at Riverton, N. J., in 1918, that old lotus plants practically all disintegrate during the winter.

Coquillett (*4, p. 154*) lists the following parasites of *Pyrausta penitalis: Exorista vulgaris* Fall.; *Hypostena variabilis* Coq.; *Panzeria penitalis* Coq.; *Phorocera comstocki* Will.

Full-grown larvae of this species are larger and more robust than either *Pyrausta nubilalis* or *P. ainsliei*. The pupae are also larger but the adults are smaller than the former, almost equaling in size the moths of the latter. The larvae measure 22.5 mm. or 0.87 inch in length, and are heavily pigmented. They usually are brownish-black on the dorsum and white ventrad. The head capsule is larger than in either of the above forms and has a mottled appearance, owing to the collection of the pigment in small regular areas, leaving the remainder of the chitin light colored. In *ainsliei* the pigmentation of the head is uniformly dark colored. However, in *nubilalis* specimens of the larvae can be found intermediate between *ainsliei* and *penitalis:* in such cases the pigment collects into irregular blotches. Heinrich sepa-

rates *penitalis* from *nubilalis*, as follows:

Epicranial puncture O^a lying postero-dorsad of ocellus VI; mandible longer than broad; distal tooth concaved—*P. penitalis*.

Epicranial puncture O^a lying directly posterior to ocellus VI; mandible square, distal tooth pointed—*P. nubilalis*.

(Pl. 2, I, J.)

PHLYCTAENIA RUBIGALIS GUENÉE

Scopula rubigalis Guen., 1854, Delt. et Pyr. (Bdv. et Guen., Hist. Nat. Ins. Lépidop.) 8: 398.
Botys oblunalis Lederer, 1863, Wien. Ent. Monatschr. 7: 372, 469.
Botys harveyana Grote, 1877, Canad. Ent. 9: 104.
Phlyctaenia ferrugalis auct. (nec Hübner).
Pionea rubigalis Guen. Hampson, 1899, Proc. Zool. Soc. London, 1899: 242.
Phlyctaenia rubigalis Guen., Chittenden, 1901, U. S. Dept. Agr., Div. Ent. Bul. 27: 7; Slingerland, 1901, N. Y. Cornell Agr. Exp. Sta. Bul. 190: 159; Busck and Heinrich, 1925, Jour. Agr. Research 29: 140.

Phlyctaenia rubigalis Guen. (the greenhouse leaf-tyer) is apparently the most widespread and troublesome insect pest with which greenhouse operators have to contend. It is especially troublesome where chrysanthemums are exclusively grown. In New England practically every greenhouse examined in 1920–21 was severely infested by this insect. Although it is primarily a pest of chrysanthemums, it has also been found to attack rose, violet, snapdragon, and geranium. Davis (*5, p. 100*) found in Illinois that chrysanthemum and spearmint were particularly subject to attack. Infestations have also been found in celery, cabbage, beets, lettuce, cauliflower, and strawberry among garden crops. Many more plants, both ornamentals and vegetables, not mentioned here, doubtless are fed upon by the larvae of this species.

In 1921 the moths appeared in numbers in chrysanthemum houses the first week in May. From this time until the blooms and plants were cut in October and November the insect bred continuously. During the months of July, August, and September it was possible, on examination of greenhouse chrysanthemums, to find the insect in all stages of development—eggs, larvae, pupae, and moths. It was practically impossible to follow the broods in the field on account of the overlapping of generations.

After the blooms are marketed in October and November growers cut the old plants back severely, dumping or burning the tops out of doors. The old plants thus pruned soon give forth shoots which are used for the new crop of chrysanthemums the subsequent season. This dumping or burning of the tops reduces in a large measure the infestation, since practically all the developing larvae and pupae are removed from the house. Moreover, the moths flying in the houses have no further place for oviposition, and since it requires a week to 10 days for the old plants to produce shoots the majority of the insects die and the house is rid of the pest. In houses where a variety of ornamentals is grown the insect breeds continuously through the entire year; it is equally abundant in winter and summer. Davis (*5*) noted that the insect was rarely found in greenhouses during the winter months and attributed the fact to the cooler temperatures prevailing in houses in the winter. In the spring, greenhouses are apparently reinfested from moths emerging from the old chrysanthemum tops or litter out of doors, gaining entry through the ventilators. In midseason in 1921 it required 35 days under glass for the insect to complete its development from egg to adult. The larvae usually pupate in the webbed-up leaves.

The larvae (pl. 1, F) for the most part are surface feeders—a few instances have been noted in market gardens and from specimens received at the Arlington laboratory of the entry of the larvae into the stems of celery—feeding on the under surfaces of the leaves of chrysanthemum and also webbing the leaves together with strands of silk, forming thereby an irregular fabrication in which they live. They have also been observed to feed on the leaf buds in the axils of the expanded leaves and on the flower buds, which on opening produce imperfect blooms. When the larvae are numerous this latter type of feeding greatly discounts the market value of the blooms but has no serious effect on the vitality of the plant.

Full-grown larvae slightly resemble the European corn borer in a superficial way. The distribution of the setae on the body is almost identical in both species. Both are pyralids. The larvae measure when full grown 0.68 inch in length. They are pale sage-green in color and strongly translucent, so that portions of the trachea, particularly about the spiracles, may be seen through the cuticula in living specimens. Average specimens bear a dark green stripe on the median dorsal plane and two white broader stripes halfway between the former and the pleura. The head capsule is pale yellow and mottled; the thoracic and anal shields are light yellow. The chitinized areas about the tubercles or

pinacula are pale (*10, p. 142*), indistinct, and sometimes wanting entirely. The chitinization of the pinacula forms the chief character whereby they can be readily separated from the larvae of the corn-borer group.

Two near relatives have been reared at the Arlington laboratory: *Phlyctaenia tertialis* Guen. from elderberry, in which it breeds and overwinters as a larva, and *Phlyctaenia terrealis* Tr. from carnations. The larva of the latter enters the stem of the carnation plant through the crown of terminal leaves. Its presence is readily detected by the wilting of this section of the plant which growers pinch off and unfortunately throw in the walks of their greenhouses. The larvae soon desert these pinched-off parts and return to the living plants to reinfest them. This wilting bears much resemblance to the wilting in certain plants resulting from the attack of the European corn borer, but the latter is not known at this time to infest carnation.

DIATRAEA ZEACOLELLA DYAR

Diatraea zeacolella Dyar, 1911, Ent. News 22: 203; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer. No. 5437.

Previous to Dyar's separation and description of *Diatraea zeacolella* (the larger cornstalk borer) in 1911 entomological writers on economic subjects referred to this insect as *Diatraea saccharalis* Fab., believing the species injuring corn identical with the one doing damage to sugar cane.

The larger cornstalk borer is primarily a southern insect. The records by various workers, especially Leiby (*17, p. 10*), show that it extends from Delaware to Florida, west to Louisiana, and possibly occurs in Kansas, Oklahoma, and Texas. It passes through two complete generations, one in the spring and early summer and the other in late summer and early fall. The overwintering larva pupates in the early spring.

The principal food plant of the insect is the corn plant. Other food plants have been recorded as: Gama grass, sorghum (sorgo), sugar cane (Howard), probably Johnson grass, and Guinea corn (Ainslie).

On hatching from the eggs the larvae feed on the epidermis of the leaves of corn (*17, p. 28*), finally working their way into the unopened whorl of corn leaves which on expanding contain a rather uniformly sized series of holes running across the breadth of the leaf (*19, p. 8*). Another series of irregular perforations in the leaf may also be present when it fully expands, owing to the fact that the larvae feed within the bud in an irregular way, horizontally or upward, or downward. After feeding in either fashion for a time the larvae desert this part of the corn, migrating downward and reentering the plant generally between the first and second joints. Since the insect has two broods and the nature of the feeding for the two generations of larvae is closely parallel, much real injury is caused to growing corn, the growth of the plant being impaired and production being consequently diminished. The larvae apparently never attack the grain on the ear as do those of the European corn borer. "The larger cornstalk borer habitually passes the winter in the taproot of the corn plant, whereas *nubilalis* may be found at any point in the stalk and in the ear" (Caffrey).

Although the larger cornstalk borer possesses many structural characters common to the European corn borer, the two larvae differ quite radically in appearance. The larvae of *Diatraea zeacolella* are of two types, a summer and a winter form. The essential difference between the two types consists in the pigmentation of the pinacula. In the summer form they are brown to black, well defined, in strong contrast to the white body and honey-yellow head. In the winter form these areas have all faded out, are pale, indistinct and seem to fuse with the whiteness of the body. The larvae average 24.9 mm., or almost an inch in length. They are, therefore, slightly longer than *nubilalis*, and more robust.

The supracoxal pinaculum on the mesothorax in Diatraea bears two setae, No. VI [7] (pl. 2, C); in *nubilalis* this same area is unisetose, seta VI (pl. 2, A) (*15, pl. 4, fig. 1*).

[7] Larval chaetotaxy is a difficult study. It is particularly difficult to trace setal homologies. The writer makes no pretense of being well informed on the subject. The Roman numerals employed in designating setae follow the system of Dyar, who did the pioneer work in America on this subject. Heinrich's papers have been freely consulted and studied. Fracker (*10*) has instituted a nomenclature of Greek letters, believing that by its utilization one can more readily grasp the homologies of setae. It seems that Fracker established his homologies by plotting one segment above the other. He failed, however, to take note of the fact that the hairs occur on definite body areas and that their changes in position are caused by modifications of the areas themselves. It has been pointed out clearly by Heinrich that when hairs are absent, as frequently happens on the ninth abdominal segment, it is necessary to trace the folds to determine what body areas have been reduced or crowded aside before one can decide what particular seta is missing. Fracker did not do this and, therefore, frequently has given the wrong designation to his seta. Fracker's system apparently would have been, in many ways, an improvement over the Roman method if he had correctly worked out the homologies between the segments.

NOMOPHILA NOCTUELLA DENIS AND SCHIFFERMÜLLER

Tinea noctuella D. & S., 1776, Syst. Verz. Wien., p. 136.
Pyralis hybridalis Hübn., 1796, Eur. Schmett., Pyr., 29, pl. 17, fig. 114.
Nephopteryx indistinctalis Walk., 1863, Cat. Brit. Mus., pt. 27: 59.
Botys helvolalis Maassen, 1890, Stübel's Reise, p. 170.
Nomophila noctuella D. & S., Dyar, 1902, List N. Amer. Lepidop. No. 4342; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer. No. 5012.

Nomophila noctuella (the celery stalk worm) has been known for a long time, yet notwithstanding its abundance and wide distribution, very few references to it occur in the literature on economic entomology. It is cosmopolitan, occurring in Europe, Algeria, Bengal and the eastern and western parts of the United States.

In 1893 Felt published a paper on this species (7) in which he records the fact that the larvae feed on certain grasses. He also states that Leach in "British Pyralids" records the larvae from *Polygonum aviculare* in Scotland. The interest of the writer in *Nomophila noctuella* is due to its occurrence as a larva on celery in the market gardens in the vicinity of Arlington, Mass. It was found frequently associated with the larvae of *Pyrausta nubilalis* for which it has been mistaken.

Field observations in New England in 1920–21, supported by insectary rearings, indicate that there are two complete generations, the insect passing the winter as a larva in litter. Felt states that in New York there seem to be three broods a year. At Arlington moths emerged in 1921 during the last week in May, the larvae from which, forming the first generation, pupated and emerged over a 3-week period, from July 7 to July 22. The second-brood larvae, for the most part, were mature the third week in September and passed the winter as such. The pupal stage of the first brood was of 13.8 days duration in 1921.

The eggs, 0.37 mm. in diameter, are globular, slightly compressed at either pole, and sculptured. The sculpturing appears much like that on noctuid eggs—a series of radiating prominent ridges from the poles connected to each other by secondary transverse striae which increase in length in the region of the greatest diameter of the egg. The eggs are a dull white and slightly iridescent, this iridescence disappearing as incubation proceeds. At hatching time the eggs are brown, owing to the brown head capsule and thoracic shield of the larva within. The eggs are laid in groups varying from 4 to 48, but are in nowise attached to one another. They are usually to be found on the under sides of the leaves of the plant.

The larvae (pl 1, H) were taken commonly from celery on the grooved side of the celery stalk, and also apparently preferred the blanched and semi-blanched portions of the stalk. Only the outermost stems of the plant were observed to be infested. Since these are discarded when the celery is shipped to market it is doubtful whether the species does perceptible damage to celery. The larvae make irregular, shallow excavations on the stalk, usually covering the area with strands of silk, although instances were found where the feeding was carried on without any silken inclosure over the feeding area. They are surface feeders, never drilling into or tunneling the stalk as is so characteristic of the European corn borer. The larva on being prodded moves forward or backward with equal rapidity.

Full grown larvae measure 0.81 inch in length. They are a pale green or dirty white in color, depending apparently upon what they have been eating. The latter type is invariably found on celery. The head capsule and thoracic shield, as well as the large nearly quadrate areas about the tubercles on the median plane of the back, are chestnut brown. The larvae are distinguished from those of the corn-borer group by the unusually long setae or hairs arising from the pinacula on the segments of the body; in *Pyrausta nubilalis* these setae are comparatively short and stiff. Seta III of the eighth abdominal segment is fully three times the length of the same seta in the European corn borer. Another difference readily noted is in the arrangement of the pinacula on the median plane of the back. These areas are close to those of the opposite half of the larva, and each posterior pair on the abdominal segments follows its antecedents in the same horizontal plane. In the Pyrausta larvae discussed, the posterior median pair of pinacula are latero-ventrad of horizontal lines drawn through the anterior pair; the four median pinacula on each abdominal segment if connected to one another by an imaginary line would describe a trapezoid on Pyrausta larvae, a parallelogram on those of Nomophila.

LOXOSTEGE SIMILALIS GUENÉE

Nymphula similalis Guen., 1854, Delt. et Pyr. (Bdv. et Guen., Hist. Nat. Ins. Lépidop.) 8: 403.

Nymphula rantalis Guen., 1854, Delt. et Pyr. (Bdv. et Guen., Hist. Nat. Ins. Lépidop.) 8: 405.
Ebulea murcialis Walk., 1859, Cat. Brit. Mus. pt. 18, p. 746.
Scopula crinisalis Walk., 1859, Cat. Brit. Mus., pt. 18, p. 798.
Botys siriusalis Walk., 1859, Cat. Brit. Mus., pt. 18, p. 563.
Botys licealis Walk., 1859, Cat. Brit. Mus., pt. 18, p. 563.
Botys nestusalis Walk., 1859, Cat. Brit. Mus., pt. 18, p. 784.
Scopula thoonalis Walk., 1859, Cat. Brit. Mus., pt. 18, p. 785.
Scopula dictimealis Walk., 1859, Cat. Brit. Mus., pt. 18, p. 785.
Nephopteryx intractella Walk., 1863, Cat. Brit. Mus., pt. 27, p. 55.
Botys posticata G. & R., 1867, Trans. Amer. Ent. Soc. 1: 22.
Scopula occidentalis Pack., 1873, Ann. Lyc. Nat. Hist. N. Y. 10: 260.
Botis communis Grote, 1876, Canad. Ent. 8: 99.
Loxostege similalis Guen., Dyar, 1902, List N. Amer. Lepidop., No. 4354; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., No. 5025.
Pyrausta caffreii Flint and Malloch, 1920, Bul. State Ill. Dept. Reg. & Educ., Div. Nat. Hist. Surv. 13: 304, figs. 43, 44 (vide Heinrich 2, p. 57).

The larva of *Loxostege similalis* (the garden webworm), is a foliage depredator, feeding exclusively in the open or in an irregular fabrication formed by webbing leaves together with silk. It is not a borer as is the European corn borer, yet it resembles the former in appearance. The greatest resemblance is structurally, the distribution of the setae on the thoracic and abdominal segments being most alike. The insect occurs in most of the Central, Western, and Southern States, but can also be found in Mexico and South America. It is particularly damaging in California, Nebraska, Iowa, Missouri, New Mexico, Kansas, Oklahoma, and Texas. In the three last-named States second and third annual cuttings of alfalfa have been entirely destroyed in certain years.[8]

The garden webworm, apparently incorrectly named since it injures to a large extent cereal and forage crops, feeds on and injures beets, sugar beets, potatoes, corn, cotton, wheat, alfalfa, and, doubtless, garden crops. Its natural food appears to be *Amaranthus* sp. (pigweed), and *Chenopodium* sp. (lamb's-quarters). Since the year 1909 the injury to alfalfa has steadily increased, becoming more serious and widespread.

A striking difference between this larva and those of the corn-borer group is the habit which the caterpillar of Loxostege has, when full grown, of descending from its food plant to the surface of the soil, where it forms a small silken cocoon in the litter and within this it pupates. The European corn borer, as a rule, pupates within its burrow and never forms a defined cocoon.

The larva is green-brown in color and three-fourths to seven-eighths of an inch in length. The pinacula on the dorsum are strongly chitinized, brown to black, and prominent; those on the pleura are pale and not so strongly chitinized. In Pyrausta larvae heretofore discussed the pinacula on the various parts of the body are uniform with respect to one another in color and chitinization; there is no distinction between those of the dorsum and those on the pleura. In *nubilalis* Seta IV+V on the ninth abdominal segment almost equals Seta III in size; in *similalis* IV+V is small and vestigial (pl. 2, A, E).

In the latitude of Kansas and Oklahoma the insect apparently passes through four generations, whereas in Texas it seems to breed continuously throughout the year, summer and winter.

FAMILY OLETHREUTIDAE

EPIBLEMA SCUDDERIANA CLEMENS

Hedya scudderiana Clemens, 1860, Proc. Acad. Nat. Sci. Phila. 12: 358.
Euryptychia saligneana Clemens, 1865, Proc. Ent. Soc. Phila. 5: 141.
Paedisca affusana Zeller, 1875, Verh. Zool.-Bot. Ges. Wien 25: 307.
Paedisca scudderiana Kellicott, 1882, Canad. Ent. 14: 161; Walsingham, 1884, Trans. Ent. Soc. London, 1884: 140.
Eucosma scudderiana Clemens, Fernald, 1902, Dyar, List. N. Amer. Lepidop., No. 5139; Kearfoot, 1905, Proc. U. S. Nat. Mus. 28: 354; 1905, Canad. Ent. 37: 208; Barnes and McDunnough, 1917, Check List Lepidop. Bor Amer., No. 7014.
Epiblema scudderiana Clemens, Heinrich, 1923, U. S. Nat. Mus. Bul. 123: 147, fig. 271.

Epiblema scudderiana (the Bidens borer) is common throughout eastern Massachusetts and doubtless occurs in equal numbers over the North Atlantic States. Specimens have been received at the European Corn Borer Laboratory from Maine, New Hampshire, Massachusetts, Connecticut, New York, New Jersey, Pennsylvania, Ohio, and Michigan. With the exception of two records, one occurrence in lamb's-quarters (*Chenopodium* sp.) and one from burdock (*Arctium* sp.) the insect has been collected only from beggar-ticks (*Bidens frondosa*) and goldenrod (*Solidago* sp.).

Field observations indicate that the insect is single brooded in Massachusetts. In 1921 moths appeared the third week in June, the overwintering

[8] Information as to the distribution, food plants, and seasonal history was obtained from Farmers' Bulletin 944 (*16*).

larvae pupating the first week and throughout the month of June and early July. Young larvae were found as early as June 28 in Bidens plants. The majority of the larvae in the field were full grown the third week in September, 1921. The larvae remain over the winter in their food plants. The character of the injury to Bidens by Epiblema coincides to a considerable degree with that caused by the European corn borer to the same weed. The diameter of the tunnels or burrows in the plant is smaller, however, and the frass extruded therefrom is never in so large a quantity, nor does it adhere to the plant beneath the entrance to the burrows, usually collecting on the soil at the base of the plant in small cone-shaped heaps.

The yellowish-white caterpillars (pl. 1, A) bear a superficial likeness to the immature stages of the European corn borer. Since both species infest Bidens and since the larvae of the two bear prominent dark-colored pinacula, confusion of the species is possible. On close examination of the larvae, however, one readily sees that the structural characters are different. The single ranked crochets on the pseudopods are arranged on the planta in a completed circle, whereas those of the Pyraustinae are of three ranks and are arranged in an imperfect circle or imperfect ellipse, sometimes referred to as a penellipse with the opening to the horseshoe outermost. This olethreutid bears three setae on the prespiracular pinaculum, whereas pyralid and noctuid larvae bear only two setae on this structure. There is no pigmented band or stripe on the median dorsal plane of the larvae of *Epiblema scudderiana*. Other less evident differences exist, such as the shape of the mandible, the structure of the maxillae, and the setal arrangement on the head capsule.

EPIBLEMA STRENUANA WALKER

Grapholita strenuana Walk., 1863, Cat. Lepidop Het. Brit. Mus., 28: 383.
Grapholita exvagana Walk., 1863, Cat. Lepidop. Het. Brit. Mus., 28: 383.
Steganoptycha flavocellana Clemens, 1865, Proc. Ent. Soc. Phila., 5: 138.
Grapholitha subversana Zeller, 1875, Verh. Zool.-Bot. Ges. Wien 25: 318.
Paedisca strenuana Walk., Wals., 1879, Illus. Lepidop. Het. Brit. Mus., 4: 52; 1884, Trans. Ent. Soc. London, 1884: 140.
Eucosma strenuana Walk., Fernald, 1902, Dyar, List N. Amer. Lepidop., No. 5129; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., No. 6981.
Eucosma minutana Kearfott, 1905, Proc. U. S. Nat. Mus. 28: 356; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., No. 6982.
Eucosma antaxia Meyrick, 1920, Exot. Microlepidop. 2 (*11*): 344.
Epiblema strenuana Walk., Heinrich, 1923, U. S. Nat. Mus. Bul. 123: 140, figs. 257, 258.

The larve of *Epiblema strenuana* (the ragweed borer) has been taken only from *Anbrosia artemisiaefolia* L. (ragweed) (*14, p. 141*). Apparently it is not common in eastern Massachusetts. It differs from the previous species of Epiblema in having the pinacula on the thorax and abdominal segments feebly pigmented; the setae are pale yellow; the thoracic and anal shields pale; the head light brown in lieu of black. It can be distinguished from the European corn borer and other pyralids by the characters enumerated under *scudderiana*.

FAMILY OECOPHORIDAE

DEPRESSARIA HERACLIANA DE GEER

Phalaena heracliana De Geer, 1771, Mém. Ins. 2: 407.
Phalaena heraclei Retz., 1783, De Geer, Gen. et Spec. Ins., p. 45.
Pyralis umbellana Fab., 1794, Ent. Syst., pt. 2, 3: 286.
Haemylis pastinacella Dupon., 1836, Hist. Nat. 8: 153.
Haemylis umbellella Zett., 1840, Ins. Lapp., p. 999.
Depressaria ontariella Bethune, 1869, Canad. Ent. 2: 3.
Depressaria heracliana De Geer, Dyar, 1902, List N. Amer. Lepidop., No. 5889; Busck, 1902, Proc. U. S. Nat. Mus. 24: 748; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., No. 1478.

Depressaria heracliana (the parsnip webworm) is a European species imported into this country probably in the late sixties of the last century. It was, at any rate, first noticed in 1873 as of economic importance, and has since distributed itself over the northern portions of the United States, west and south to the State of Mississippi and in Canada. It has been named the parsnip webworm from the habit which the larva possesses of webbing up the flower and seed heads of the parsnip. The insect breeds on wild carrot and probably on other umbelliferous plants.

The larvae (pl. 1, E) of this species were observed in 1921 at Cliftondale, Mass. They were found living in masses and feeding on the flower heads of parsnip, which they had webbed together with strands of silk. In time this nestlike structure becomes dirty and foul from the collection of excreta. When the larvae were one-half to three-fourths grown, they were observed gradually to desert their feeding quarters externally and enter the parsnip stalk, although some remained in the original web and pupated therein. The majority, however, tunneled through the cortex and thin white medulla of the

plant, feeding in groups on the latter tissue, which lines the hollow stem. The larvae cover their entrances through which they pass into the stalk with tympana of silk. Later the moths rupture these structures as they emerge from the stalk. When full grown, the larva spins a pad of silk against the inside wall of the stem, in which one finds the cremaster of the pupa securely fastened, thus suspending the larval head downward. In 1921, under insectary conditions, the insect remained as a pupa 12.7 days. The moths appeared in mid-July and continued to emerge into August.

Full-grown larvae are slightly smaller than the larvae of *Pyrausta nubilalis*, to which they bear resemblance. The black head, thoracic shield, and pinacula of the body segment sharply contrast with the yellowish-white integument of the larva. The anal shield is lemon yellow. The dorsal surface is granulated, but the skin granulations are never as dense as in the larvae of Pyrausta. The prespiracular pinacula bear three setae, the chitinous rings of the spiracles are stout and black, the crochets on the planta of the pseudopods are in a completed circle, and the setal arrangement on the ninth abdominal segment differs from that of all the larvae previously discussed in this paper (pl. 2, D).

FAMILY NOCTUIDAE

HELIOTHIS OBSOLETA FAB.

Bombyx obsoleta Fab., 1793, Ent. Syst., pt. 1 3:456.
Noctua armiger Hübn., 1810, Samml. Eur. Schmett., Noct., 370.
Heliothis armiger Hübn., Dyar, 1902, List No. Amer. Lepidop., No. 2300.
Chloridea armigera Hübn., Hampson, 1903, Cat. Lepidop. Phal. Brit. Mus., 4:45.
Heliothis obsoleta Fab., Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., No. 1090.

Although the larva of the corn earworm (*Heliothis obsoleta* Fab.) primarily attacks the grain on the ear of field and sugar corn, it is also known to feed freely on the silk and sparingly on the foliage. The European corn borer attacks all parts of the plant. It habitually lives within burrows made in the stalk, in the midrib of the leaf, on the ear, and in the cob. The corn earworm never drills through the tough fibrous cortex of the corn plant or into the cob. When the insect attacks cotton, however, the larva bores into the boll, destroying the lint and the seeds. Both the corn borer and the earworm feed on the corn kernels, but the larva of the latter invariably enters at the tip end, whereas the corn borer enters the ear at every conceivable point, such as through the tip, through the side, and through the butt, and very frequently, indeed, it lives in the pith of the cob. Caffrey has noted that the breaking over of tassels in a cornfield does not necessarily preclude feeding in these parts by the corn borer, since he has found that the earworm is capable of occasionally doing the very same thing, particularly in late-developing sweet corn. The corn earworm is a most important destructive pest. Estimates have been made of an annual loss of from 2 to 5 per cent of the total corn crop produced in the United States, or of $30,000,000 to $50,000,000. The insect occurs throughout the United States and in many parts of the world.

In addition to the injury inflicted by the earworm to corn, it attacks such important crops as tobacco and cotton. Tomatoes, beans, forage plants, and many others are periodically damaged. The caterpillars are large, robust forms belonging to the same family of insects as the cutworms. They are exceedingly variable in color, ranging from green to rose, brown to black, striped, spotted, or plain. Thus in a field one may collect larvae which to the layman or to the farmer would seem entirely different. When the larvae have completed their growth they fall or descend of their own accord to the soil, burrow 2 to 5 inches into it, and construct cells wherein they pupate. The corn borer never enters the soil to pupate but transforms in its burrow or in another place of seclusion to which it has migrated. In the Gulf States there are four broods of the earworm annually, along the Gulf coast there may be five or six, whereas in the northern States there are two, with possibly only one in the province of Ontario, Canada. In Massachusetts in 1921 the corn earworm was abundant and destructive.

The larvae are larger than those of the European corn borer, measuring when fully grown 1¼ to 1½ inches in length. The dorsal pinacula are small, pale, or black, depending on whether the larva is light or dark colored; the supraspiracular pinacula on the pleura are always black or deep brown. The crotchets on the prolegs are characteristically noctuid (pl. 2, M) in a mesoseries. In the European corn borer, as pointed out elsewhere, the crochets are arranged in an incompleted circle with the opening outermost. The head capsule of the corn earworm is yellow-brown and mottled, whereas in the corn borer the head capsule is usually

black or a deep chestnut brown and is always black as the larva emerges from the egg. Just as the larvae of the corn borer group have only two setae, IV and V, on the prespiracular pinacula of the prothorax, so on this point the noctuids are structurally identical, but on the third, fourth, fifth, and sixth abdominal segments the setal distribution differs with respect to IV and V; IV is removed quite a distance from V and is directly caudad of the spiracle (pl. 2, H).

ACHATODES ZEAE HARRIS

Gortyna zeae Harris, 1841, Ins. Mass. Inj. Veg., p. 319.
Achatodes zeae Harris, Dyar, 1902, List N. Amer. Lepidop., No. 2158; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., No. 2642.

Since Harris described *Achatodes zeae* (the spindleworm) and the injury to corn caused by its larva, infrequent references have been made to it in entomological literature. Forbes in his classical Twenty-third Report gives an account of it (*9, p. 85*) and characterizes it as the spindleworm, apparently from the fact that it has been found attacking corn in the so-called spindle stage of that plant.

The species is very abundant everywhere in Massachusetts in elderberry, but has only rarely been seen attacking corn. Dyar (*6, p. 174*) gives its distribution as the North Atlantic States. On May 28, 1921, eight young larvae were found in an equal number of sweet corn plants, but this corn was not advanced to the spindle stage. The corn was in proximity to a large growth of elderberry. The larvae found in the stalks were in well-defined burrows and were headed upward in these tunnels. The latter are very similar in appearance to those of the European corn borer, but in the above case the seasonal occurrence of Achatodes larvae did not synchronize with the latter. The plants were badly wilted as if from Papaipema attack.

The natural food plant of this species is elderberry in which the larvae have been found in large numbers. On May 17, 1921, it was possible to cut out, from new elderberry shoots springing from the soil, quantities of the larvae. On June 10 they were found in all stages of development. The first pupae were taken in the burrows June 16 and subsequently some were found which pupated in the soil, but pupation occurs largely in the burrows where the larvae developed. The pupae are large, reddish-brown and are supplied with two prominent processes on the cephalic end.

The larva (pl. 1, G) is striking in appearance. Head, thoracic and anal shields, and the pinacula are glossy black; the body of the larva is yellowish-white. On all the abdominal segments, with the exception of the ninth, the two anterior median pinacula are larger than those of the caudal pair; the anterior pair are circular in contour, the caudal pair elliptical. On the ninth abdominal segment the anterior set equals the posterior pair in size.

The larva of this species may be distinguished from other larvae treated in this paper by an examination of the anal shield. This is strongly chitinized, black, rugose, and bears on its caudal margin three pairs or a row of prominent and strongly produced spines.

KEY TO THE SEPARATION OF LARVAE TREATED HERETOFORE

1. Larvae without strongly produced spines on the anal shield 2
   Larvae with strongly produced spines on the anal shield ..... *Achatodes zeae*
2. Larvae with three setae on prespiracular shield 3
   Larvae with two setae on prespiracular shield 4
3. Setae III and V on prespiracular shield small, indistinct, much smaller than Seta IV ..... *Epiblema scudderiana*
   Setae III and V on prespiracular shield prominent, well defined, equal to Seta IV in size ..... *Depressaria heracliana*
4. Crochets on planta of pseudopods arranged in an incomplete circle or penellipse 5
   Crochets arranged otherwise; in mesoseries ..... *Heliothis obsoleta*
5. Seta III of the eighth abdominal segment normal sized; pinacula bearing Setae VII and VIII, respectively, on the eighth abdominal segment wanting, poorly defined or only lightly chitinized 6
   Seta III of the eighth abdominal segment abnormally long, terminating short of the caudal border of the ninth abdominal segment; pinacula bearing Setae VII and VIII, respectively, on the eighth abdominal segment well defined, strongly chitinized ..... *Nomophila noctuella*
6. Pinacula on body segments pale and indefinite (*D. zeacolella*, winter form) 7
   Pinacula on body segments strongly chitinized, well defined, and heavily pigmented 8

7. Supracoxal pinacula on the mesothorax furnished with one seta (Seta No. VI)---*Phlyctaenia rubigalis*
   Supracoxal pinacula on mesothorax furnished with two setae (Seta No. VI)---*Diatraea zeacolella*
8. Seta IV and V on the subdorsal pinaculum of the ninth abdominal segment small and nearly obsolete, this pinaculum bearing one large seta which is III---*Loxostege similalis*
   Seta IV and V on the subdorsal pinaculum of the ninth abdominal segment large, almost equal in size to III---9
9. (*11, p. 178*) "Epicranial setae and puncture A^1, A^2, and A^a lying in a straight line or with A^a somewhat postero-laterad of A^2, not postero-dorsad"---10
   "Epicranial setae and puncture A^1, A^2, and A^a forming an obtuse angle with A^a postero-dorsad of A^2---*P. ainsliei*
10. "Epicranial puncture O^a lying postero-dorsad of Ocellus VI; mandible longer than broad; distal tooth concaved"---*P. penitalis*
    "Epicranial puncture O^a lying directly posterior to Ocellus VI; mandible square; distal tooth pointed"---*P. nubilalis*

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THE INFLUENCE OF TEMPERATURE ON THE INFECTION AND DECAY OF SWEET POTATOES BY DIFFERENT SPECIES OF RHIZOPUS [1]

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INTRODUCTION

Temperature is regarded as an important factor in the infection and decay of sweet potatoes. Rather specific instructions regarding the proper temperatures at which sweet potatoes should be stored are given in the various publications dealing with storage. The temperatures recommended for curing and storing vary somewhat, but in general are as follows: For curing, 26° to 32° C.; for storing, 10° to 14°. Notwithstanding these recommendations, there is little information available regarding the relation of temperature to infection and decay by the various organisms responsible for the decay of sweet potatoes.

Among the most important organisms which cause this decay are some of the species of Rhizopus. The following species were found by Harter, Weimer, and Lauritzen (*3*)[2] to be capable of decaying sweet potatoes: *R. tritici* Saito, *R. nodosus* Namysl., *R. maydis* Bruderl., *R. oryzae* Went and Pr. Geerligs, *R. delemar* (Boid) Wehmer and Hanzawa, *R. arrhizus* Fischer, *R. reflexus* Bainier, *R. artocarpi* Racib., and *R. nigricans* Ehrnb.

Hanzawa (*1*) studied 14 species of Rhizopus, separating them into three thermal groups on the basis of such characters as the presence or absence of spore germination and the abundance of mycelial growth, and the presence or absence of fruiting on potato plugs at various temperatures and after different periods of time. He does not give the exact increments of growth with the rise in temperature or after different periods of time. No measurements are recorded. These three groups are as follows: (1) High-temperature group, *Rhizopus oryzae, R. arrhizus* Fischer, *R. chinensis* Saito, *R. japonicus* Vuillemin, *R. tonkinensi* Vuillemin, and *R. batatas* Nakazawa; (2) intermediate-temperature group, *R. nodosus, R. tritici, R. kasanensis* Hanzawa, *R. trubini* Hanzawa, and *R. usamii* Hanzawa; and (3) low-temperature group, *R. nigricans.*

Weimer and Harter (*7*) made an intensive study of the germination of spores in sweet potato decoction and of the mycelial growth and fructification on potato agar of 11 species of Rhizopus. They likewise separated the species studied into three thermal groups. Their grouping is slightly different from Hanzawa's and includes some species not studied by him and excludes others.

The work of these authors resulted in the following classification: (1) High-temperature group, *Rhizopus chinensis;* (2) intermediate-temperature group, *R. tritici, R. nodosus, R. delemar, R. oryzae, R. arrhizus,* and *R. maydis; and* (3) low-temperature group, *R. nigricans, R. microsporus* v. Teig., *R. reflexus,* and *R. artocarpi.*

Their results show the maximum, optimum, and minimum temperatures for spore germination, mycelial growth, and fructification, the increments of growth with the rise in temperature and increments of growth after different periods of time.

One difference between the two groupings is that Hanzawa places *Rhizopus oryzae* and *R. arrhizus* in the high-temperature group, while Weimer and Harter place them in the intermediate. This discrepancy is apparently not serious, for if Hanzawa's data are examined, one finds as good reason for placing these species in the intermediate group as with *R. chinensis;* in fact, a better reason if one eliminates *R. delemar,* which Hanzawa in his table still retains under *R. oryzae* and which shows a wider temperature range for sporulation than does *R. oryzae. R. chinensis* is sufficiently different from all the other species in its temperature response to place it in a group

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[2] Reference is made by number (italic) to "Literature cited," p. 810.

by itself, especially when it is considered in connection with the species Weimer and Harter (*7*) employed in their experiments.

The experiments recorded in the present paper had for their purpose (1) the determination of the optimum, maximum, and minimum temperatures for the infection and decay of sweet potatoes; (2) the measurement of the amount of decay with rise in temperature and after different periods of time; and (3) the determination of the time required for infection to take place, by six species of Rhizopus. These species are, *R. tritici*, *R. oryzae*, *R. maydis*, *R. reflexus*, *R. artocarpi*, and *R. nigricans* strains *a* and *b*.[3]

The reason for eliminating three of the species (*Rhizopus nodosus*, *R. delemar*, and *R. arrhizus*) found by Harter, Weimer, and Lauritzen (3) to be parasitic on the roots of sweet potatoes, and employing only the remaining six species, will now be considered. *R. tritici* and *R. nodosus* are almost identical in their temperature responses on culture media (*7*). The temperature responses of *R. oryzae* and *R. delemar* are likewise almost identical according to the data of Weimer and Harter (*7*) and there is scarcely any difference according to Hanzawa's data (*1*), excepting in fruiting, and here the difference is not great. Since these two pairs likewise behaved similarly in infection experiments (*3*), it was decided to eliminate one of each pair. *R. arrhizus* was not available at the time these experiments were conducted. *R. maydis* was selected because it is rather distinct in some of its characters, particularly in that it produces very few spores.

Rhizopus nigricans, *R. reflexus*, and *R. artocarpi* belong to the same thermal group, but show some variation in their temperature responses (*6*, *7*). For example, there is a slight variation in the maximum, minimum, and optimum temperatures for spore germination, mycelial growth, and fructification of these three species (*7*). *R. artocarpi* is more successful in competition with *R. nigricans* at 14° C. than at 12°, and *R. reflexus* is more successful at 12° than at 14° (*6*).

Rhizopus nigricans is by far the most important organism of the group from the standpoint of the amount of decay it causes (*6*); in fact, in storage, it causes most of the soft rot decay of sweet potatoes.

MATERIALS

Single-spore strains of the above listed species were used in these experiments. The variety of sweet potatoes used was Little Stem Jersey. The potatoes were cured for 10 days at from 25° to 30° C. and stored at temperatures ranging mostly between 10° and 15°.

In these experiments it is important that the size and shape of the potatoes should be as nearly uniform as possible, otherwise any irregularities at the surface of the potatoes around the point of infection would alter the amount of decay. To be comparable in any two cases, the decay must be able to proceed within equal radii, which it can not do for any length of time if the potato is small and irregular in shape. It is not possible to obtain potatoes of identical size and shape, but it is possible to approach this condition sufficiently to make the results comparable. If one is comparing the amount of decay at several temperatures, there must be enough tissue available for the decay to proceed at the higher temperatures over sufficiently long periods to give opportunity for decay to take place at the lower temperatures.

APPARATUS

The apparatus described by Lauritzen and Harter (*6*) was used throughout these experiments.

Four types of experiments form the basis of this work, the methods employed varying with the type.

FIRST TYPE OF EXPERIMENT

This type of experiment was designed to determine the time required for infection, where infection depends on the organisms normally present on the potatoes. The potatoes in the condition in which they were received from the storage house, were wounded[4] to the same degree on the side of the roots at the point of the greatest diameter.

It is believed that wounding is the chief predisposing condition permitting infection by Rhizopus, and the methods employed in these experiments enable one to obtain some idea of the time required for infection to take place. They

[3] Strain as used here merely means that the organisms used were obtained from different sources and isolated from different hosts. Strain *a* was isolated from tomato by F. C. Meier, and strain *b* from sweet potato by one of the writers.

[4] The wounding was done with a special instrument with two blades, 1 cm. long and 0.2 cm. wide. The blades were mounted parallel to each other 1 mm. apart on a brass rectangular plate 1.5 by 1.5 cm., which served as a shoulder. On the opposite side of the plate was a handle used to shove the blades into the potatoes. The blades were pushed into the potatoes twice, as far as the shoulder permitted, the second time at right angles to the first.

at least permit of a comparison of the time required for infection at the various temperatures, because the wounding was the same at all temperatures and all the potatoes were grown and stored under identical conditions. These experiments were conducted the latter part of December and the first part of January so that there had been opportunity for some accumulation of spores on the potatoes, there being more spores (*6*) on the potatoes with the advance of the storage season.

the potatoes were wounded as in the first type of experiment and inoculated by dipping them in a spore suspension made from an equal number of cultures of *Rhizopus tritici* and *R. nigricans* (strain *a*).

Potatoes of uniform size and shape were selected, washed in tap water, dried in the laboratory for 24 hours, and divided into lots according to size and shape, each lot being weighed, wounded, and then inoculated by dipping them in a spore suspension.

TABLE I.—*Time required for the infection of sweet potatoes by Rhizopus tritici and R. nigricans at various temperatures*

Temperature (°C.)	Time required for infection	Number of potatoes inoculated	Number of potatoes infected	Remarks
32.5	43 hours	20	9	Size of lesions varied from 1 to 6 cm. in diameter. No infection in 24 hours.
27.5	do	20	7	Size of lesions varied from 0.5 by 1 to 4 by 5 cm. in diameter. No infection in 24 hours.
25.5	do	20	19	Size of lesions varied from 0.3 by 1 to 4 cm. in diameter. No infection in 24 hours.
23.5	do	20	14	Size of lesions varied from 1 to 3 cm. in diameter. No infection in 24 hours.
19.5	do	20	14	Infection just started. No infection in 24 hours.
18.0	do	20	6	Do.
15.0	3 days	20	12	Infection just evident. No infection evident after 2 days.
13.5	4 days	20	15	Lesions from 1 to 2 cm. in diameter.
9.0	7 days	20	20	Lesions varied from 3 to 10 cm. in diameter. No infection evident in 5 days.

Table I shows the number of days required for infection to take place at the various temperatures by *Rhizopus tritici* and *R. nigricans*. At temperatures of 18° C. and above infection took place in 43 hours and less. At 18° and 19.5° infection had just started in 43 hours. Above these temperatures infection started earlier, but in no case as early as 24 hours. At 15° three days were required for infection, and at 9° five to seven days. The time required for infection to take place at any of the temperatures is comparatively short. There will be some variation from this time, depending upon the degree of wounding, the time of season, and the conditions under which the potatoes are stored. When infection has once been established the time required for the potatoes to decay completely is comparatively short, four or five days at most. If potatoes are to be utilized after they have been badly wounded, they must be consumed within the first few days.

SECOND TYPE OF EXPERIMENT

These experiments were devised to measure quantitatively the amount of decay at various temperatures, where

After having been subjected to this treatment the potatoes were placed at the various temperatures and left for infection to take place and decay to develop. After the decay had developed to a suitable degree, the lots were weighed again, the decay removed by a spoon or some other blunt instrument, and the remaining undecayed portion weighed. The weight of the decay was obtained by subtracting the latter weight from the first weight. The reason for making the second weighing was to ascertain the loss or gain of water during the infection and incubation periods. There was a slight loss of water, especially at the higher temperatures, which was correlated to some extent with the amount of decay. There was a very slight gain of water at the lower temperatures in some cases where the humidity was exceptionally high. The humidity, however, was fairly comparable throughout the chambers, being slightly lower at the higher temperatures. The variation in loss of water was not sufficient to alter the general relations obtained.

It will be seen from the results recorded in Table II that the amount of decay increases rapidly with the rise in temperature, more rapidly than one

would expect from the van't Hoff law. The quantity of decay varies from nearly six times to many times as much at one temperature as at 10° below, except between 20.5° and 21.5° C. and at temperatures near the upper limit for infection.

TABLE II.—*Amount of decay at various temperatures by Rhizopus tritici and R. nigricans*

Temperature	Time after inoculation	Number of potatoes inoculated	Number of potatoes decayed	Weight of decayed tissue [a]
° *C.*	*Days*			*Grams*
37.0	4	50	48	3,511
32.5	4	50	46	5,906
29.0	4	50	42	4,600
26.5	4	50	29	2,626
21.5	4	50	37	925
20.5	4	50	40	885
14.5	7	50	34	1,878
11.5	7	50	43	254
6.5	12	50	50	680
4.0	12	50	50	109

[a] These weights were calculated on the basis of 30 potatoes from the decayed potatoes at each temperature.

There was less decay between 20.5° and 21.5° C., but these temperatures correspond to the lower limit of infection for *Rhizopus tritici* when this method of wounding and inoculation are used (*6*), and there is often a decrease of decay observed at these temperatures. It is not clear whether this is due to some factor associated with the pathogens or to resistance on the part of the host.

The increments of decay per degree are particularly high at temperatures between 11.5° and 14.5° C. and between 4° and 6.5°.

No isolations were made from these potatoes, but previous experience (*6*) would justify one in concluding that the organisms involved were *Rhizopus tritici* [5] and *R. nigricans*, *R. tritici* exclusively at 30° C. and above, and *R. nigricans* at 20° and below, the two overlapping between 20° and 30°.

THIRD TYPE OF EXPERIMENT

The procedure in these experiments was the same as in the second, except that the "well" method (*2*) of inoculation was employed. Forty-eight-hour old cultures of the organisms used, grown on 2½ c. c. of sweet potato decoction in test tubes at room temperature (20 to 25° C.), were introduced into "wells" 1 cm. in diameter and 4½ cm. deep. The "wells" were deep enough so the inoculum reached approximately the center of the potatoes. It was desired to start infection at or near the center so that the decay might proceed for some time before reaching the surface or skin of the potato as it proceeded outward from the point of infection. An indeterminable error results if the decay is permitted to reach the surface, because as soon as it reaches the skin at any point it stops in that direction, thus limiting the amount of decay.

From the data presented in Tables III and IV the six species studied can be separated into two groups according to their temperature responses: First, a high temperature, and second, a low temperature. *Rhizopus tritici*, *R. oryzae* and *R. maydis* belong to the former and *R. nigricans*, *R. reflexus* and *R. artocarpi* to the latter.

This relation is illustrated in Figures 1 and 2. Curves representing all of the organisms except *Rhizopus oryzae* are shown in Figure 1. *R. oryzae* and *R. tritici* are shown in Figure 2. The curves in Figure 1 were drawn from weighings (Table III) of decay at different temperatures after two days and those in Figure 2, from weighings made after three days. It will be seen from Figure 2 that *R. oryzae* belongs to the same group as *R. tritici*. The optimum temperatures for infection for the three species of the high temperature group lie between 32° and 35° C. (Table III and figs. 1 and 2), and those for the three species of the low temperature group between 18.5° and 23° C. (Table III and fig. 1).

In case of the high temperature group there is a close correspondence to the optimums obtained by Weimer and Harter (*7*) (*R. tritici*, 33° to 35° C., *R. oryzae*, 31° to 34°, and *R. maydis*, 30.5° to 32.5°, for mycelial growth on culture media. The optimum temperatures obtained by Weimer and Harter (*7*) for *R. nigricans* (23° to 26°), *R. reflexus* (26° to 28°), and *R. artocarpi* (26° to 28°) are somewhat higher than the optimum temperatures for infection obtained in the present experiments (*R. nigricans*, strains *a* and *b*, 18.5° to 23.5°, *R. reflexus*, 18.5° to 23°, and *R. artocarpi*, 23° to 24° (Table III). The data in connection with the last three species correspond to those recorded by Lauritzen (*5*) for *Puccinia graminis* Pers. var. *tritici* Erick. on wheat and *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. on beans, where the optimum temperatures for infection were slightly lower than optimum temperatures for germination of spores and growth of mycelium.

[5] *Rhizopus tritici* here is used in a collective sense and may include *R. nodosus*, *R. oryzae* and *R. delemar*. It is not possible from present knowledge to distinguish morphologically between any of these species

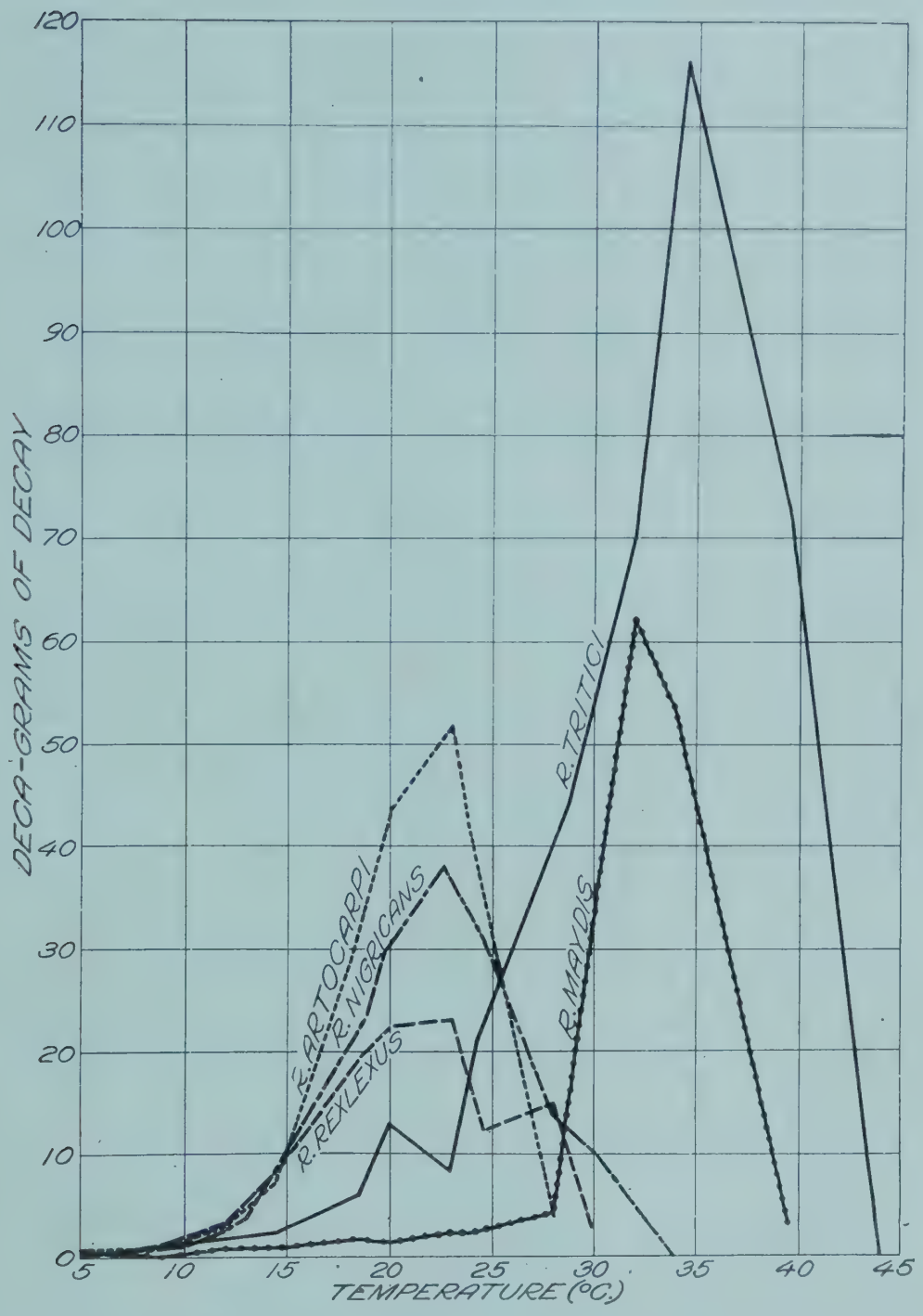

FIG. 1.—Curves showing the increments of decay by five species of Rhizopus with the rise in temperature after two days

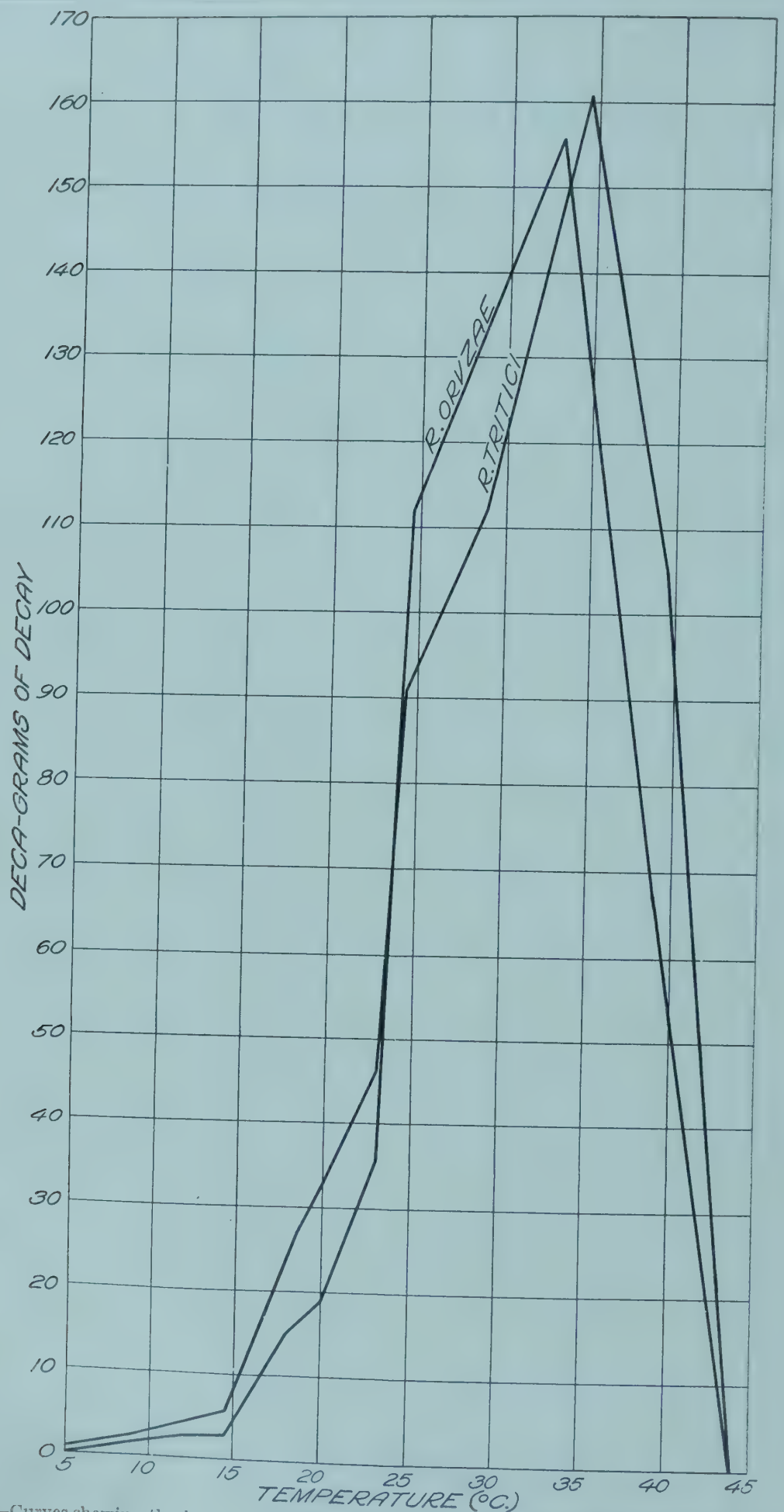

FIG. 2.—Curves showing the increments of decay by *Rhizopus tritici* and *Rhizopus oryzae* with the rise in temperature after three days

TABLE III.—*Amount of decay of sweet potatoes in grams produced by six species of Rhizopus at various temperatures and after different periods of time* [a]

Temperature	Rhizopus tritici					Rhizopus oryzae			Rhizopus maydis		Rhizopus nigricans strain *a*			Rhizopus reflexus		Rhizopus artocarpi	
	1 day	2 days	3 days	5 days	6 days	1 day	3 days	9 days	1 day	2 days	2 days	4 days	5 days	2 days	3 days	1 day	2 days
°C.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
39.5	155	729	1,051						22.5	31							
39.0						164.5	660										
34.5	212	1,162	1,607											6	0		
34.0									91	534							
33.0						228.8	1,555									0	
32.0	222	702	1,399						40.5	624						3.5	
29.8														28			
28.8	61	443	1,121								0	6	0				
28.0						122.2	1,295		21	45				149.1	100	43	40
24.5						77.7	1,119				36	245	526	122.5	191		
24.3	50	211	909														
24.0									17	23						135	408
23.0	23.5	83	462	1,196	888	72	356		18.5	25	36.1	578	770	231	273	85	516
20.0	32	127	329	1,176	698	24	188		11.5	12	79	362	264	224.1	348	68	432
18.5	39	62	272	780	890				13.5	16	72	508	635	194.5	650		
18.0						19.6	151									40	281.1
14.5		25	56	321	415	4	27.7		7	8				82.6	352.	7	73
14.3											20	264	474				
12.0						0	25	275	11	8	15	242	321	33.5	231	4	32
9.0		12	24	68	72	0	16	20.0	7		7	27	59	12.1	56	0	10
5.0			8	14	10	0	0	5.5	0	7		0	0	0	12.5	0	9

[a] The data recorded in Table III are the average of two or more experiments of all the species (strain *a* of *Rhizopus nigricans*), except that of *R. maydis* where only one experiment was conducted, due to the difficulty of obtaining sufficient spores for a spore suspension from which to inoculate the tubes of sweet potato decoction. The weighings given represent the decay of four potatoes and in most instances they are the averages of two or more experiments. In some instances more than four potatoes were used, but the weighings given were calculated on the basis of four potatoes as a unit.

In general, it might be expected that the optimum temperature for infection would fall below the optimum for growth on culture media, because of the resistance offered by the hosts and the direct availability of the food in the culture media.

It has been noticed that at a temperature of 33° C. and above a larger percentage of unwounded sweet potatoes normally decay with Rhizopus soft rot than at lower temperatures. In other words, the resistance to Rhizopus is apparently broken down at the higher temperatures. This may explain why the optimum for decay by *R. tritici R. oryzae* and *R. maydis* is as high as for growth on artificial media.

Figures 3, 4, 5, 6, 7, and 8 show temperature curves of the six species indicated (*Rhizopus nigricans*, strain *a*) and represent the amount of decay after different periods of time. It will be noted from these curves (see also figs. 1 and 2) that the amount of decay does not increase in the same proportion as the rise in temperature in the early stages of decay in case of any of the organisms, there being a lag at the lower temperatures. There is a partial recovery of this lag with the lapse of time. The additional time required for the fungus to become established at the lower temperatures before decay actually begins may be a factor in producing the lag.

TABLE IV.—*Amount of decay of sweet potatoes caused by Rhizopus nigricans strain b, two days after inoculation* [a]

Temperature	Weight of decay	Number of potatoes inoculated	Number of potatoes decayed
°C	*Grams*		
38		20	
34		20	
30	103.2	37	12
28	138.0	47	28
24.5	312.0	47	40
22.5	379.6	47	39
19.7	296.0	47	42
18.8	234.4	46	43
13.0	38.4	47	47
11.8	24.0	47	47
10.0	9.2	47	46
7.0	5.2	46	46
5.0		47	

[a] In this table the results of two experiments are given and the weighings are calculated on the basis of four potatoes as in Table III. Controls were run in connection with these experiments. Sterile sweet potato decoction was introduced into the "well" instead of cultures of the organisms. The potatoes remained sound in all cases, indicating that the decay in case of the inoculated potatoes was due to the organism employed in the inoculum. Further proof of this indication is the fact that the decay of the respective organisms was always within their temperature growth limits on culture media (7).

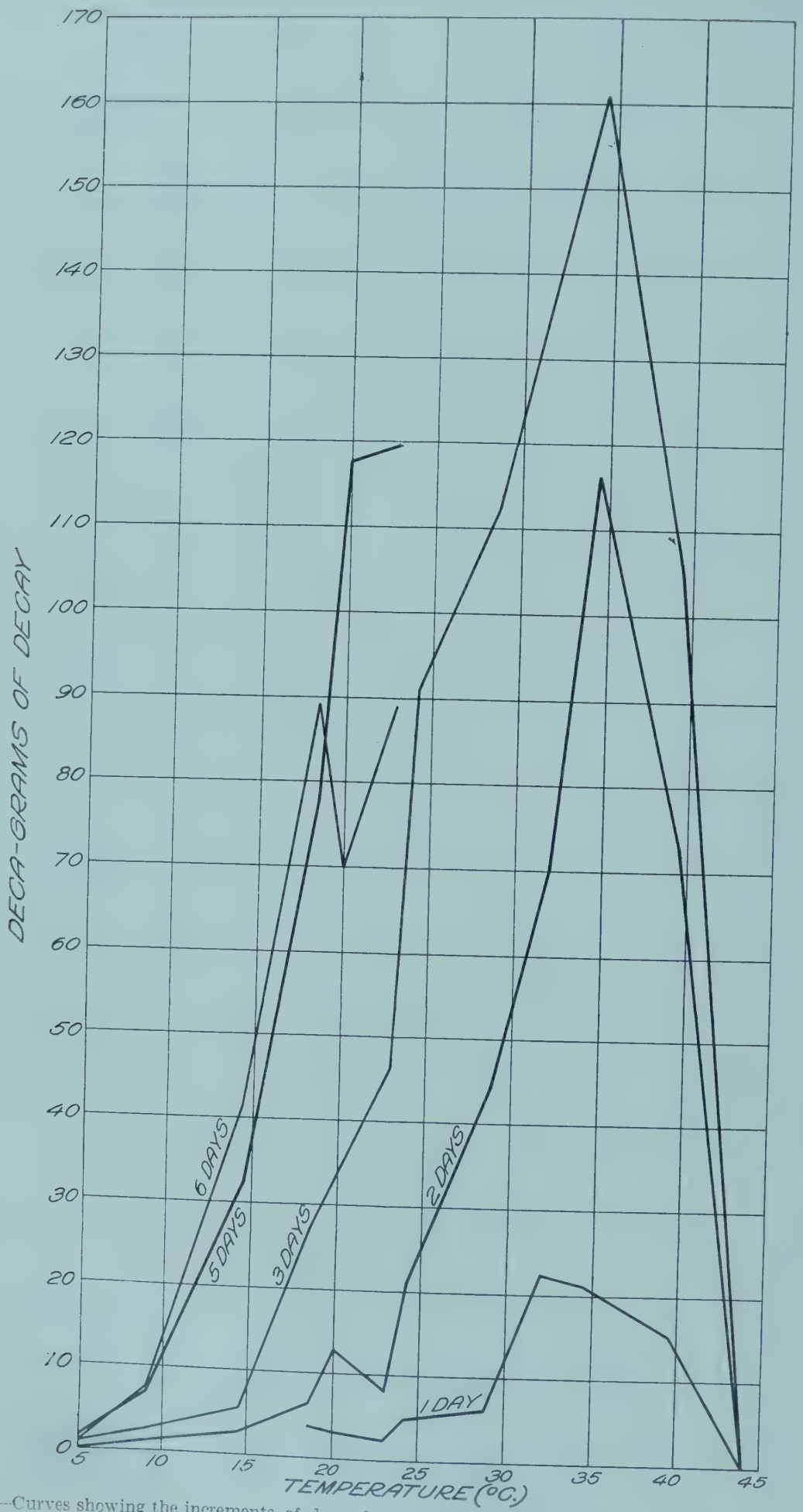

FIG. 3.—Curves showing the increments of decay by *Rhizopus tritici* with the rise in temperature after different intervals of time

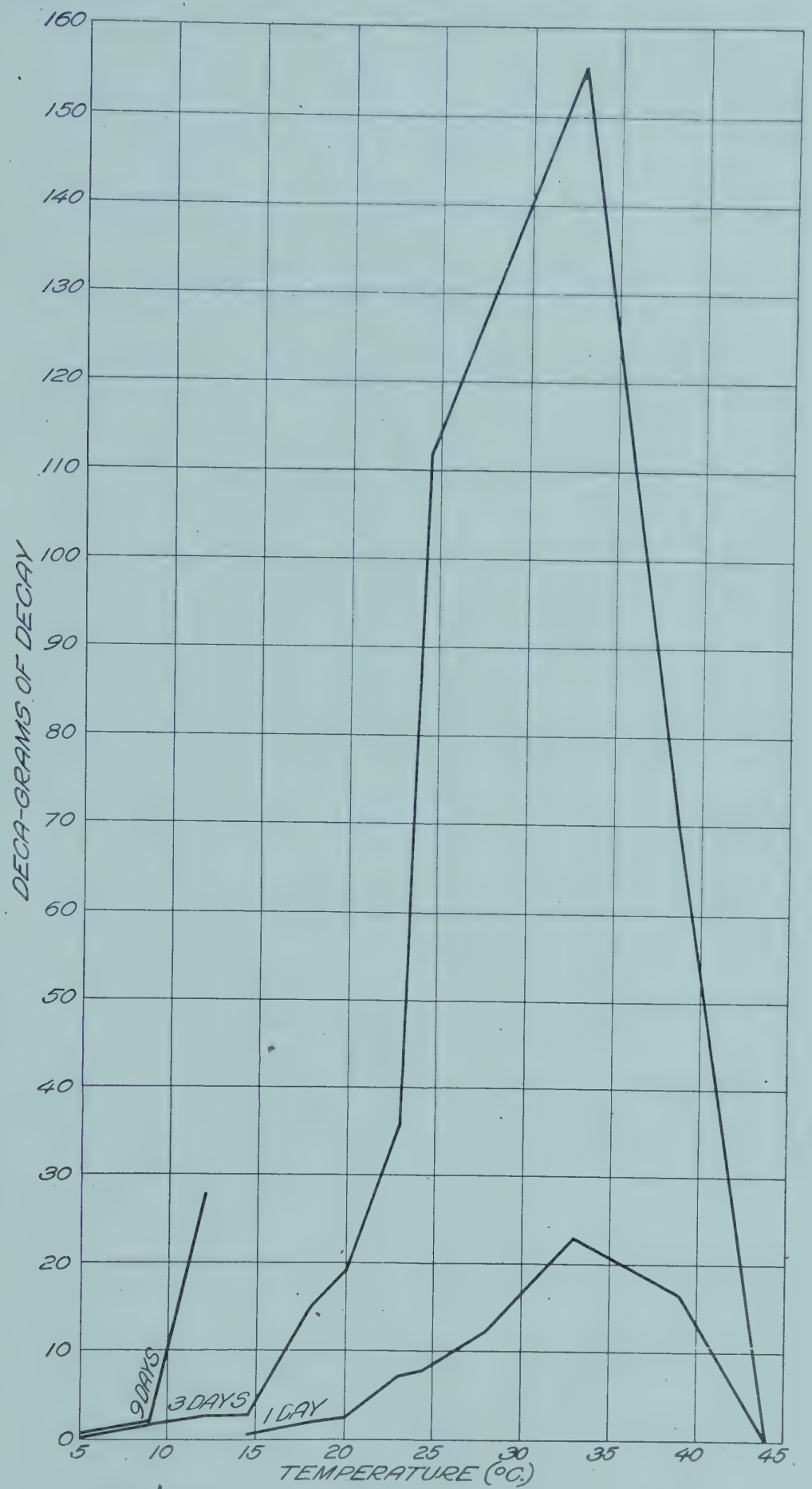

FIG. 4.—Curves showing the increments of decay by *Rhizopus oryzae* with the rise in temperature after different intervals of time

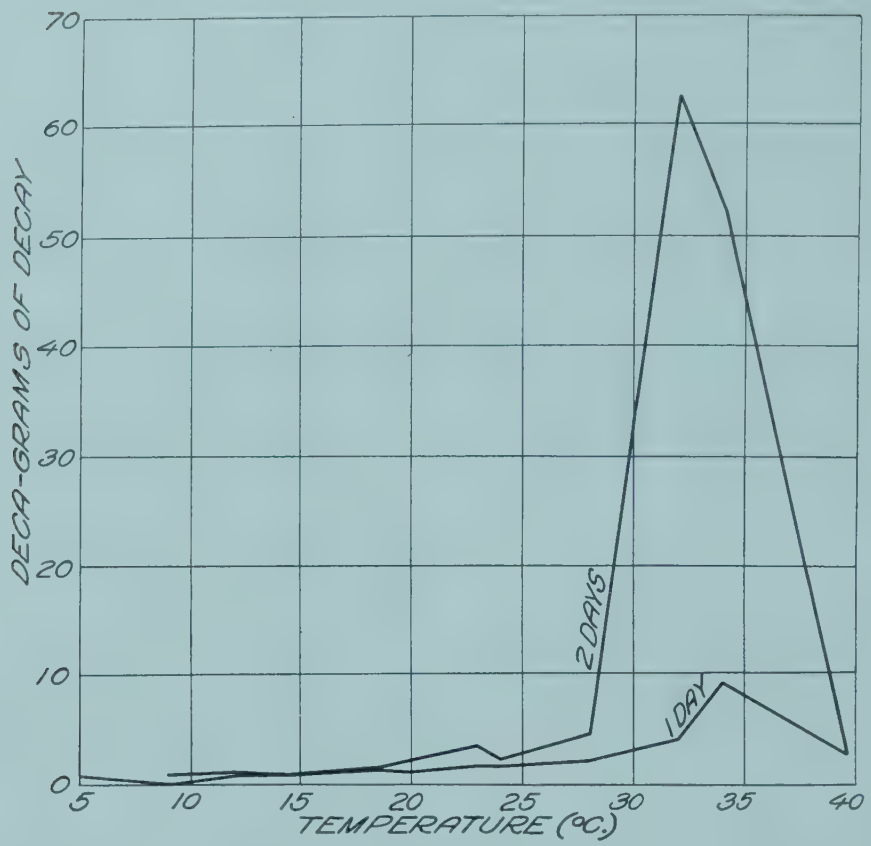

FIG. 5.—Curves showing the increments of decay by *Rhizopus maydis* with the rise in temperature after different intervals of time

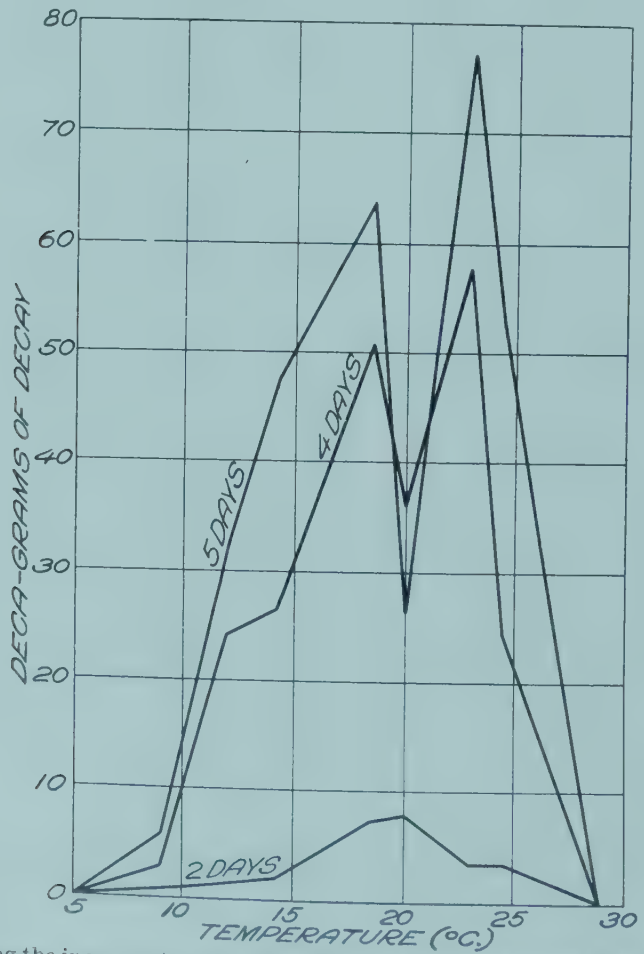

FIG. 6.—Curves showing the increments of decay by *Rhizopus nigricans* strain *a*, with the rise in temperature and after different intervals of time

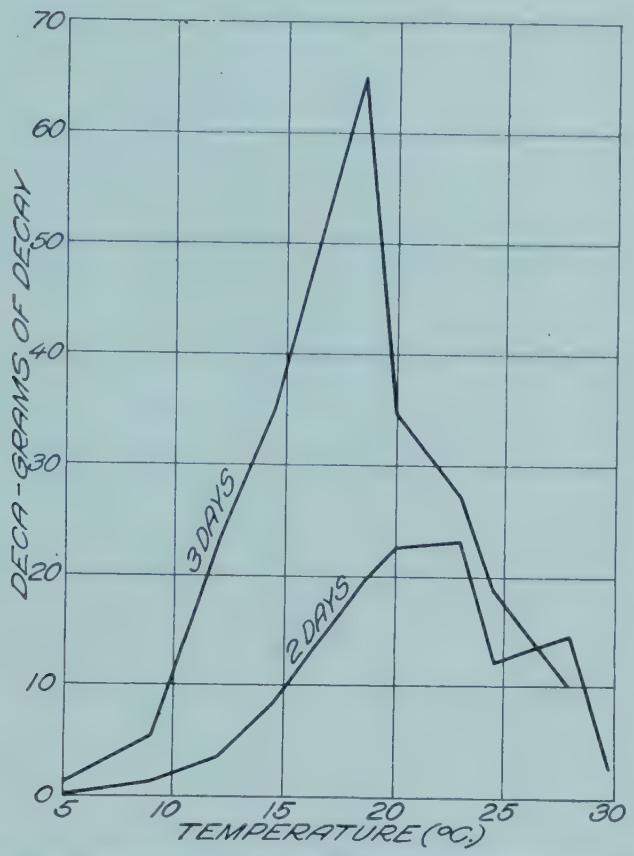

FIG. 7.—Curves showing the increments of decay by *Rhizopus reflexus* with the rise in temperature and after different intervals of time

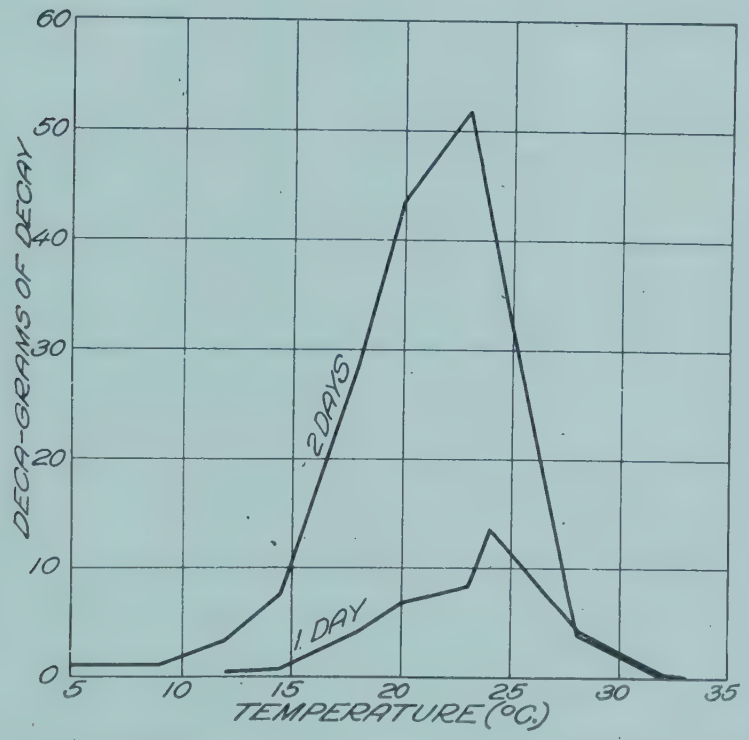

FIG. 8.—Curves showing the increments of decay by *Rhizopus artocarpi* with the rise in temperature and after different intervals of time

The amount of decay during the first day is much less than in the succeeding days, as would be expected. This may be accounted for (1) because some time is required for the fungus to become established on its new medium, and (2) because the operating surface increases rapidly with the lapse of time. Theoretically, because of the increasing operating surface, each succeeding interval of time should show an increased amount of decay. The data (Table III and fig. 9) in connection with *R. tritici* show this tendency, but not consistently. This inconsistency may be explained in part by the fact that in the later stages of decay there is a definite limit because of the size of the potatoes. would seem to indicate that the method employed here will give reasonably accurate results. It is true that some of the curves show some decided breaks, but the results taken as a whole are fairly consistent. It is not expected that they would be so uniform as those obtained in culture media, for here there are two organisms to be dealt with instead of one. Erratic behavior, so far as infection and the amount of decay in individual potatoes is concerned, is often observed. More uniform results probably would be obtained if a larger number of potatoes were employed, but the time element in setting up a particular experiment then becomes a source of error.

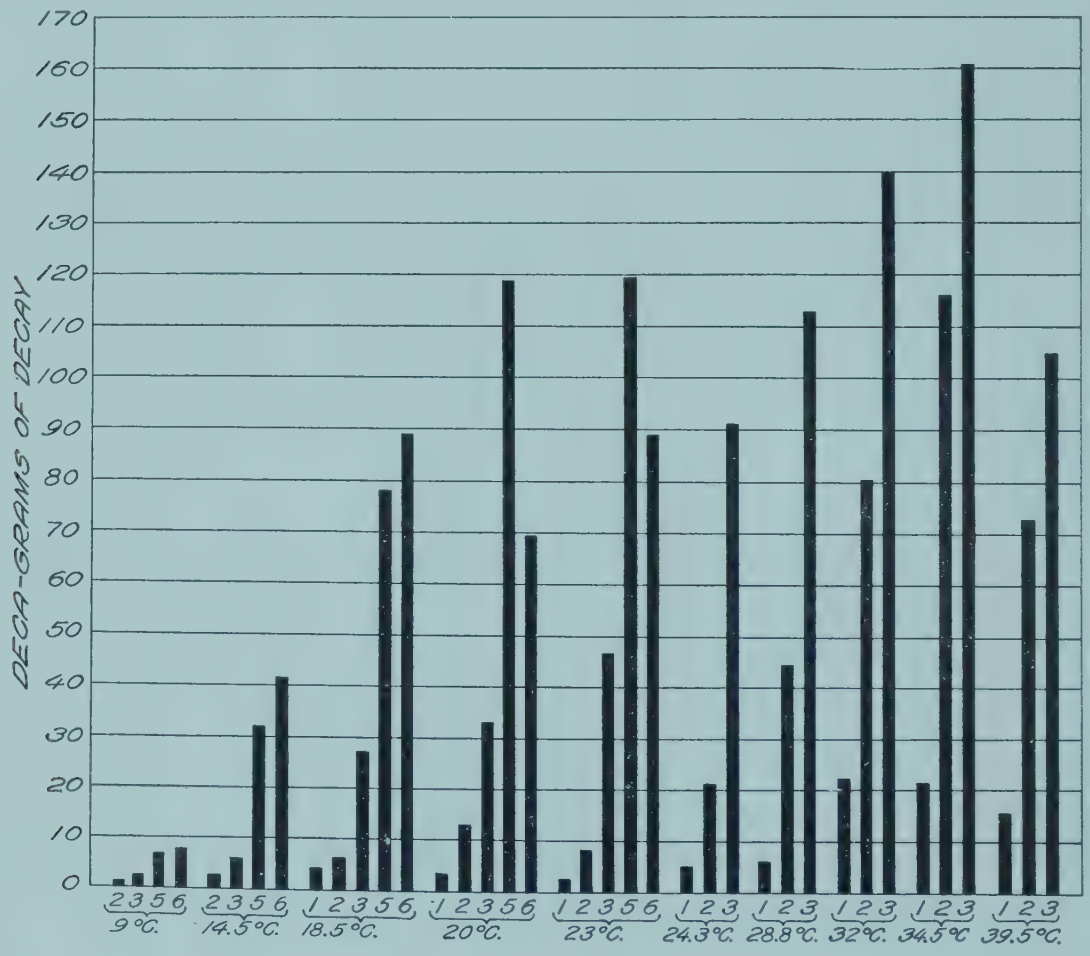

FIG. 9.—Graph showing the amount of decay by *Rhizopus tritici* at various temperatures and at different intervals of time

The extreme variation in the optimum temperatures given for the successive periods of time for any of the species is as follows: *Rhizopus reflexus*, 4.5° C. (Table III and fig. 7); *R. nigricans*, 3° (Tables III and IV and fig. 6); *R. tritici*, 2.5°, but is identical in two cases (Table III and fig. 3); *R. maydis*, 2° (Table III and fig. 5); *R. artocarpi*, 1° (Table III and fig. 8); and *R. oryzae*, 0° (Table III and fig. 4). This fact

The increase in the amount of decay with the rise in temperature is consistently high throughout these experiments as well as in the second type of experiment, higher as a rule than where other methods of measurements are employed, but it represents more nearly the actual loss involved than where the diameter of a spot is used as a measurement of the amount of decay; and even where the decay is a surface

phenomenon the loss due to the disease is more nearly represented by the area covered by the spot than by the diameter.

FOURTH TYPE OF EXPERIMENTS

These experiments were devised to determine the upper and lower temperature limits at which infection by the various organisms will take place, where the "well" method of inoculation was used.

UPPER TEMPERATURE LIMITS.—The upper temperature limits for infection by *Rhizopus tritici*, *R. oryzae*, and *R. maydis* are recorded in Table V, and for *R. nigricans*, *R. reflexus*, and *R. artocarpi* in Table III. *R. tritici* was isolated from each of eight potatoes at 42° C. but was not isolated at 44°. The upper limits found by Weimer and Harter (*7*) for mycelial growth were 42° to 45.5°.

any infection in the experiments in which it was used was 28.8° C. (Table III). Some infection took place by strain *b* at 30° after two days' time, but none at 34° (Table IV). This latter strain is the same as strain 4652, employed by Harter and Weimer in their physiological studies of strains of *Rhizopus nigricans* (*4*). They did not obtain growth on culture media of this strain at 30° in one day, but did obtain germination of spores at 31°.

Here is a case where the maximum temperature for infection exceeds that of mycelial growth on culture media, but it is entirely possible that the difference in the time element is sufficient to account for this apparent discrepancy especially since a vigorous culture (48 hours old) was used in the infection experiment as contrasted with a spore suspension drop in the mycelial growth experiment. It is possible, too, that the sweet potato

TABLE V.—*Upper temperature limits for infection of sweet potatoes by Rhizopus tritici, R. oryzae and R. maydis*

Organism with which potatoes were inoculated	Time after inoculation	Number of potatoes inoculated	Number of potatoes decayed	Number of isolations made	Temperature	Organisms isolated	
						Number of times organism used in inoculation was isolated	Other organisms isolated
	Days				°*C.*		
Rhizopus tritici	2	8	8	7	44	0	Two plates were sterile and 5 gave bacteria.
Do	2	8	8	8	42	7	1 Rhizopus tritici plus bacteria.
Do	2	8	8	8	34	8	
Rhizopus oryzae	2	8	8	6	44	0	4 plates sterile, bacteria in 2.
Do	2	8	8	7	42	3	3 plates bacteria, 1 bacteria and Rhizopus.
Do	2	8	8	8	34	7	
Rhizopus maydis	2	6	6	6	44	0	Bacteria in all plates.
Do	2	8	8	8	42	1	Bacteria in 5 plates.
Do	2	8	8	8	34	6	Bacteria in 1, 1 sterile.

Rhizopus oryzae was isolated in 3 or 4 out of 8 potatoes at 42° C., but it was not isolated at 44°. The maximum found for mycelial growth by Weimer and Harter (*7*) was from 42° to 45.5°. *Rhizopus maydis* was isolated from 1 potato out of 6 at 42°, indicating that this temperature is near the maximum. It was not isolated at 44°. The maximum temperature for mycelial growth recorded by Weimer and Harter (*7*) was 40° to 44.5°.

The highest temperature at which *Rhizopus nigricans*, strain *a*, caused

may form a better medium for growth than potato agar, the culture medium used by Harter and Weimer. It has been shown (*7*) that the maximum temperature may be affected by the culture medium.

Rhizopus reflexus caused some decay at 29.8° C. but none at 34.5° (Table III, and fig. 7). Weimer and Harter (*7*) found mycelial growth at 33°. It is possible that infection may have taken place at temperatures between 29.8° and 34.6°, but the drop in the amount of decay between 28.1°

and 29.8° was so marked as to indicate that the latter temperature is near the upper limit.

Rhizopus artocarpi caused slight, if any, infection at 32° C., considerable at 28.1°, but none at 33° (Table III and fig. 8); 32° therefore is apparently near the upper limit. It is possible that in case of the last three species the maximum might be raised some if a longer time were employed; but this seems improbable, however, for at these high temperatures if infection does not occur during the first two days the potatoes remain uninfected. Table VI contains a summary of cardinal temperatures

TABLE VI.—*Summary of the cardinal temperatures of the six species of Rhizopus studied*

Species	Minimum		Optimum	Maximum	
	Absent	Present		Absent	Present
	°C.	°C.	°C.	°C.	°C.
R. tritici		3.4	32 to 34.5	44	42
R. oryzae	5	9.0	33	44	42
R. maydis	5		32 to 34	44	42
R. nigricans (strain *a*)		3.4	18.5 to 23	28.8	24.5
R. nigricans (strain *b*)	5.0	7.0	22 to 23	34.0	30.0
R. reflexus		3.4	18.5 to 23	34.5	29.8
R. artocarpi	5	12.0	23 to 24	33.0	32.0

TABLE VII.—*Lower temperature limits for infection of sweet potatoes by Rhizopus tritici, R. oryzae, R. maydis, R. nigricans (strain a), R. reflexus and R. artocarpi*

Organism with which potatoes were inoculated	Time in days after inoculation	Temperature	Number of potatoes inoculated	Number of potatoes decayed	Number of isolations made	Organisms isolated	
						Number of times organism used in inoculation was isolated	Other organisms isolated
		°C.					
Rhizopus tritici	25	5.0	20	20	19	19	
Do	51	5.0	21	21	21	19	1 Botrytis, 1 Mucor.
Do	52	3.4	18	18	14	5	Botrytis and R. tritici mixed, 6; Botrytis, 1; bacterium, 2.
R. nigricans (strain *a*)	25	5.0	22	10	9	6	1 Rhizopus sp. and bacterium; 2 Mucor.
Do	51	5.0	20	20	20	20	
Do	52	3.4	18	18	9	3	6 Botrytis and Mucor mixed.
R. reflexus	25	5.0	21	21	16	12	4 Rhizopus sp. and bacteria. Rhizopus here probably reflexus.
Do	51	5.0	18	18	0	0	Decay too far advanced for isolation.
Do	52	3.4	18	18	18	18	
R. artocarpi	25	5.0	20	(a)	0	0	
Do	51	5.0	20	20	15	0	1 Mucor; 1 unknown organism; 10 Botrytis; 2 Penicillium; 1 bacterium.
R. maydis	51	5.0	14	14	12	0	7 Botrytis; 1 R. nigricans; 2 Mucor; 2 Mucor and Botrytis.
Do	52	3.4	18	18	12	0	8 Botrytis; 1 bacterium; 3 Mucor.
R. oryzae	51	5.0	18	17	15	0	9 Botrytis; 1 Mucor; 3 Pencillium. 2 unaccounted for.
Control	25	5.0	21	0	0		
Do	51	5.0	18	18	13		4 R. nigricans; 6 Botrytis; 3 Penicillium.
Do	52	3.4	18	13	13		2 R. nigricans; 7 Botrytis; 2 Mucor.

(a) Decay just started but dried up.

LOWER TEMPERATURE LIMITS.—The data relating to the lower temperature limits for all six species are recorded in Table VII. *R. tritici* will be seen to infect sweet potatoes readily at 5° C. by this method of inoculation, it being obtained 38 times out of 40 isolations. At 3.4° it was only obtained 5 times out of 14 isolations. This temperature is probably very near the lower limit for infection by this organism because, although Weimer and Harter (*7*) found that *R. tritici* spores germinated at 1.5°, no growth of mycelium in culture medium took place at this temperature, but mycelial growth did take place at 6.5°. No observations were made on mycelial growth between these temperatures.

There was no infection by *Rhizopus oryzae* after 51 days at 5° C. In Table III there is some evidence of infection at 5°, but the loss in weight (5.5 gm.) recorded after nine days may have been due to maceration, brought about by the enzyme that may have been present in the cultures used for inoculation and a slight amount of sweet potato decoction that was remaining in the "well." [6] Some decay (Table III) did take place at 9°. One is therefore safe in assigning some temperature between 5° and 9° as the lower temperature limit for infection. Weimer and Harter (*7*) found germination of spores at 9° but none at 7° and growth of mycelium at 11° but none at 7.5°.

It will be observed that the lower temperature limit for infection by *Rhizopus oryzae* is somewhat higher than that for *R. tritici* (Table VII). These results correspond to those obtained by Hanzawa and Weimer and Harter for spore germination, mycelial growth, and fructification (*1*), (*7*).

Rhizopus maydis caused no infection at 3.4° C. or at 5° after 51 and 52 days, respectively. Although the lower temperature limit has not been accurately determined in this case, it can be definitely said that no infection took place at and below 5°. According to the data recorded in Table III there is a wide range over which scarcely no infection takes place. What would have happened if more time (two days) had been employed can not be said. In any case the behavior exhibited is unusual. Even at temperatures from 18.5° to and including 28° scarcely any infection had taken place after two days. Weimer and Harter (*7*) obtained growth of mycelium at 7.4 but not at 1.5°.

Rhizopus nigricans strain *a* infected 26 or 27 potatoes out of 29 at 5° C. Nine of these potatoes were held 25 days and 20 for 51 days at this temperature. It infected only 3 potatoes out of 9 at 3.4° after 52 days, indicating that this temperature is near the lower temperature limit, because at the higher temperatures it was not difficult to obtain 100 per cent infection. These results are confirmed by earlier results obtained by the writers (*6*) with this strain where very slight infection was obtained at 3.5° but none below. Weimer and Harter (*7*) obtained germination of *R. nigricans* spores at 1.5° and mycelial growth at 6.5°. They give no records of mycelial growth between these temperatures.

Rhizopus reflexus infected 12 out of 16 potatoes at 5° C. Rhizopus was isolated from the other four potatoes, but being contaminated with bacteria their identity was not certain, but it seems probable that they were *R. reflexus*. Another lot showed 100 per cent infection, but the decay at the time of observation was too advanced for isolation work. At 3.4° *R. reflexus* was isolated from every potato out of 18 held at this temperature. It will thus be seen that the lower limit for infection by *R. reflexus* is probably lower than that of *R. nigricans*. Weimer and Harter (*7*) found that not only would the spores of *R. reflexus* germinate at 1.5° but the mycelium would likewise grow at this temperature.

Rhizopus artocarpi caused no infection at 5° C. Whether or not the fungus had actually established host relations at 5° and 9° in the experiments recorded in Table III can not be said definitely, because no isolations were made. Twelve degrees is the lowest temperature at which infection has been recorded, but it seems probable that it can be obtained at a lower temperature.

DISCUSSION AND CONCLUSIONS

Knowledge of the time required for infection to take place is often an important factor in the loss due to diseases. Such knowledge is especially valuable in connection with diseases of fruits and vegetables in storage and

[6] This statement likewise applies to some of the weighings recorded in Tables III and IV in connection with the other species at the lower temperatures. Where the weighings are 8 gm. and less the loss of weight can be explained on this basis. At the upper limiting temperatures the decoction dries up sooner and the action of the enzymes is less evident unless infection actually takes place.

transit, where environmental conditions are or can be under partial control. It may serve as a means of preventing infection, reducing the amount of decay, and affording a basis for predicting the amount of loss that might be expected to take place under certain conditions. Sweet potatoes exposed to cold storage conditions at temperatures between 0°C. and 5° for a period of two to six weeks will invariably become infected with *Mucor racemosus* Fes. (*6*). They can be allowed, however, to remain at these temperatures for a period of 10 days if they are removed to temperatures ranging from 10° to 15° without becoming infected with this organism.

Where sweet potatoes show some infection with black rot, it can be predicted with certainty that if they are exposed to temperatures of 18° to 30° C. the loss will be greatly aggravated. If these potatoes are held at temperatures ranging from 10° to 12° the advance of the decay will be greatly checked.

The time required for infection with Rhizopus is not only short, but there is another discouraging feature that makes it difficult and generally impossible to prevent infection where the conditions are favorable to infection. Although sweet potatoes generally must be freshly wounded to permit of infection by Rhizopus, it is only under special conditions, namely, that the wounding be shallow and clear cut and that the humidity be high, 96 to 100 per cent, that infection can be prevented after potatoes have been wounded.

It is possible to retard infection five or six days after the potatoes have been wounded (Table I) by placing them at 9° C. Losses can thus be prevented if the potatoes are consumed within this time. This period may be extended where the potatoes are not as severely wounded as they were in these experiments, which probably is usually the case. The period might also be extended if few spores were present or shortened if the spores were abundant. After infection takes place there is little hope of saving the potatoes unless they are used immediately, because the decay progresses very rapidly even at the lower temperatures.

In Table VI is given a summary of the optimum, minimum, and maximum temperatures for the six species of Rhizopus studied.

The optima for *R. tritici*, *R. oryzae*, and *R. maydis* are almost identical with those obtained by Weimer and Harter (*7*) for mycelial growth, while the optima for *R. nigricans*, *R. reflexus*, and *R. artocarpi* fell from 3° to 5° below those for mycelial growth.

This relation of the optima for infection to the optima for mycelial growth seems to be correlated with the resistance of the host which apparently breaks down at the higher temperatures.

There is a close correspondence between the temperature limits for infection and those for mycelial growth on culture media recorded by Weimer and Harter (*7*), particularly the upper limits. In some instances (*R. maydis*, *R. nigricans*, strain *b*, at high temperature and *R. tritici*, *R. oryzae* and *R. nigricans* at low temperatures) infection was obtained at temperatures beyond the limits where mycelial growth is recorded; but the next higher or lower temperatures employed in their experiments amounted to several degrees, (except in the case of *R. nigricans*, strain *b*,) and it is quite possible that had they employed intervening temperatures mycelial growth may have been obtained. Only in the case of *R. nigricans*, strain *b*, was infection obtained as high or low as the temperature recorded for the absence of mycelial growth. *R. nigricans*, strain *b*, caused infection at 30° C. in two days but did not exhibit mycelial growth at this temperature in one day. Spore germination was obtained at 31°, so that difference in time in the two instances may account for these results. *R. maydis* is the exception that does not show a close correlation between the temperature limits for infection and those for mycelial growth. It shows a wide range at the lower temperatures over which it causes no infection. The explanation of this behavior is not apparent.

The maximum temperature recorded here for infection for *Rhizopus tritici* is not quite so high as that given by Lauritzen and Harter (*6*), the former being 42° C. and the latter 44° C. It will be noted however in the latter case that *R. tritici* was isolated only three times out of 34 isolations. In any case the cardinal temperatures given are not to be regarded as definite as the figures may indicate. One must take into consideration the variations of the pathogen, host, and the experimental conditions in connection with these experiments, as well as with any physiological experiments.

The temperature range for infection by any of the low-temperature species combined with any of the high-temperature species is wide, as was

the case with *Rhizopus tritici* and *R. nigricans* (Type I) (*6*), covering temperatures varying from 3.4° to 12° C. at the lower limits to 42° at the upper. *R. tritici* has the widest range of any of the species; in fact, is as wide as the combination of any two of the low and high temperature species. It is quite possible that *R. reflexus* may be able to infect at a lower temperature than *R. tritici*, but these data do not show that it can do so.

The maximum and optimum temperatures of *Rhizopus tritici*, *R. oryzae*, and *R. maydis* are several degrees higher than those of *R. nigricans*, *R. reflexus*, and *R. artocarpi*. These six species can thus be separated into a high and low temperature group. The former group corresponds to the intermediate-temperature group of Weimer and Harter (*7*) and the latter to their low-temperature group.

The two strains of *Rhizopus nigricans* show a slightly different response to temperature. Strain *b* has a somewhat higher maximum after a given time than strain *a*. The optimum of strain *b* falls within the optimum range of strain *a*, the latter having a slightly wider range. The lower temperature limit of strain *b* was not determined except for the period of two days, which was too short to be accurate. Considerable time is required for infection at temperatures below 5° C.

The increments of decay with the rise in temperature are too large to claim any relation with the van't Hoff law, at least directly. In some instances the amount of decay is 10 times as great at one temperature as at 10° below.

Notwithstanding the fact that a comparison frequently is made between the rate of chemical reaction and the rate of growth, there seems upon careful analysis of the phenomena involved in two cases to be no real justification for such a comparison. In the first case pure chemical reactions are under consideration, while in the second numerous reactions, not all chemical, too complex for perfect analysis with present knowledge are involved.

In the present experiments the surface exposed to the action of the fungus is never the same at the various temperatures at any moment after the decay begins. The decay radiates outward in all directions from the point of infection where the "well" method of inoculation is employed, and in all directions where there is any tissue to decay where infection begins on the side of the potato forming a spherical mass of decayed tissue. The area of attack corresponds to the surface of this sphere. Not only does the formation of this sphere at the higher temperatures commence earlier than at lower temperatures but it enlarges more rapidly because of the greater rate of decay due to the effects of temperature, so that the discrepancy in the area exposed to the action of the fungus at the various temperatures becomes larger and larger with time.

Where the decay is a surface phenomenon the area exposed to the action of the fungus likewise increases with the lapse of time. In this instance the area exposed conforms more to the area of a circle instead of that of a sphere.

There is no reason to expect any correlation between linear measurements of growth and chemical reactions. In the latter case one has to do with mass; in the former, with distance. The mass in case of any decay will vary with the host and the character of the disease, in other words, where two spots are of the same dimensions in two different tissues, the mass may be totally different.

Other factors, such as the amount of enzymes secreted at the various temperatures, the passing of the hyphae of the fungus out of the area of the end products, the nature of the decay, and the character of the host and the fungus, play a part in these reactions. These factors are absent in pure chemical reactions.

SUMMARY

The data regarding the influence of temperature upon infection and decay of six species of Rhizopus are presented in this paper.

The time required for Rhizopus to infect sweet potatoes wounded but not artificially inoculated varies from five to seven days at 9° C. to 43 hours and less at 18° to 32°. This time would be expected to vary with the amount of wounding and the number of spores present on the potatoes.

The six species of Rhizopus studied can be placed into two groups according to their temperature responses, as follows: A high-temperature group, *R. tritici*, *R. oryzae*, *R. maydis*, and a low-

temperature group, *R. nigricans*, *R. reflexus*, and *R. artocarpi*. The optima for the high temperature group vary from 32° to 35° C., the maxima are about 42° and the minima vary from 3.4° to 9°; the minimum for *R. maydis* has not been definitely determined. The optima for the low temperature groups vary from 18.5° to 24°, the maxima from 30° to 34.5°, and the minima from 3.4° to 12°. The extreme temperature limits over which the entire group will infect sweet potatoes are between 3.4° and 42°. *R. tritici* will infect over this entire range. The two strains of *R. nigricans* have a slight difference in their temperature response, the one having a slightly higher maximum than the other. The increase in the amount of decay with the rise in temperature is large, being four to ten times as much as one temperature as at 10° below.

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TIME OF YEAR TO PLANT MOTHER BEETS FOR SEED PRODUCTION[1]

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INTRODUCTION

In the development of the sugar-beet seed industry for this country, the quantity and quality of seed should be among the first considerations. Some American seed companies have failed or lost heavily because of insufficient yield or poor quality of seed, which may have been due to many causes, such as poor storage, unfavorable weather conditions, poor care, or late planting. At the Salt Lake City Station (Utah) the writer has found that the quantity and quality of seed produced may vary greatly with the time of the year when the mother beets are planted. Beets planted early produce abundant seed, while those planted later produce little or no seed.

MATERIAL

Beets of the same variety and uniform as to weight, per cent of sugar, shape, and physical conditions were selected, analyzed, stored over winter, and planted during the next summer. The beets planted as late as September were apparently in as good condition as those planted in March. The apparently perfect condition of all beets at the time of planting was indicated by the fact that an unusually high percentage of the beets grew, all living beets produced seed, and there were no "trotzers," this being the reverse of conditions experienced by some investigators who have had from 50 to 90 per cent vegetative and unfruitful beets.

EXPERIMENTS

During 1922 and 1923 similar lots of beets were taken from storage each month, from March to September, inclusively, and carefully planted in rows on the same plot of ground. The soil was kept at a uniform moisture content throughout the summer. Records were kept on the physical condition and development of the plants. Data for the following physical conditions were obtained: Soil and air temperatures, soil moisture, evaporation, relative humidity, wind movement, sunlight conditions, and relative length of day. The plant was studied as to storage material, root, vegetative leaf, seed stalks, flowering, and quantity and quality of seed produced.

TABLE I.—*Average data for vegetative growth and seed production for 1922–1923*

Time of planting	Vegetative foliage	Height of seed stalks	Number of seed stalks per beets	Weight of seed produced per beets	Germination of balls
	Per cent	*Cm.*		*Gm.*	*Per cent*
Mar. 1	100	170	18	290.0	97.0
Apr. 1	90	170	18	293.0	99.0
May 1	80	125	17	228.2	96.0
June 1	40	90	12	81.0	71.0
July 1	10	80	7	40.0	50.0
Aug. 1	0	40	3	(a)	--------
Sept. 1	100	40	3	(a)	--------

[a] These did not mature.

The results given in Table I indicate that the quantity and quality of seed depend on the time of year when the beets are planted. Beets planted early produced the most and best seed. There appears to be very little difference in the results obtained from April and March plantings in these respects. The data also indicate that the quantity and quality of seed produced is directly proportional to the vegetative foliage (except for very late planting), height of seed stalks, and number of seed stalks developed during the second year. Shaw's [2] work indicates that the vegetative development is indirectly proportional to the

[1] Received for publication July 2, 1924; issued June, 1925.

[2] SHAW, H. B. CLIMATIC CONTROL OF THE MORPHOLOGY AND PHYSIOLOGY OF BEETS. Sugar 19: 387–391, 431–434, illus., 1917; 20: 23–27, 68–70, 109–112, 150–154, illus., 1918. 1917–18.

seed produced; but it appears that this is true only when dealing with beets which tend to be vegetative or are "trotzers."

The increase in production of seed in proportion to the increase in number of seed stalks per beet agrees well with the results of other investigators.[3] The increase in the number of seed stalks per beet is probably due to the inhibition of the terminal bud during the early growth period and the mobilization of an excess of available food material which gives the outer crown-buds time and favorable opportunity for development, thereby giving rise to an increased number of seed stalks. Further evidence of this is given by the fact that single or few seed stalks can be developed on beets at will. As a result there occurs the single or few seed stalk habit of annual or bolting beets, mother beets which are planted in the heat of summer, mother beets with outer crown buds destroyed due to excessive drying, and also annual or mother beets which are subjected to forced reproduction in the greenhouse at high temperatures. The factors which lead to the single or few seed stalk habit of beets are those which tend to immediate reproduction, thus forcing the terminal and retarding the outer crown buds.

Plate 1 shows that the seed spikes, seed balls, and floral bracts vary considerably with the period of year the beets are planted. Beets planted early tend to produce compact seed spikes and large seed balls, with small bracts or none. Beets planted later in the season tend to produce elongated floral stalks, smaller seed balls, and an increased number of larger seed bracts. The seed bracts on the beets planted in July and August tend toward vegetative leaves, and are much enlarged. If beets planted as late as September produce seed stalks, they are of a vegetative rosette type, as illustrated in Plate 1, E. These data indicate that later planting results in a tendency for beets to be more vegetative even when producing seed.

The rate of development of seed stalks and seed is given in Table II. The reproductive development was greatly retarded in the beets that were planted early, as indicated by the days elapsing from time of planting to time of flowering. Beets planted in the heat of summer tended to produce seed within a short period, while beets planted in the fall were very irregular in seed production and did not tend to develop reproductive organs. Photoperiodism experiments were arranged in the hope of finding some explanation of this increased seed production resulting from early planting. Both individual beets and lots of beets were exposed to the various lengths of days during spring, summer, and fall. An increased length of days during the spring had no noticeable effect upon the beets up until June 1, two months after planting. It was evident that there were other more important factors limiting the growth of reproductive organs during this period. From June 1 on, the seed stalks of beets subjected to light were probably more of a seed type, and flowered about one week earlier than beets not subjected to light. Beets planted late in the season and exposed to increased illuminations made more rapid seed-stalk growth and tended toward more reproductive seed stalks with greater per cent of late seeds. This is well illustrated by an individual beet (pl. 2, A) planted in August, the right side of which was exposed to six hours extra illumination from Sept. 8 to Oct. 15. It thus appears that light is an important factor in the lengthening of seed stalks when other limiting factors are not present.

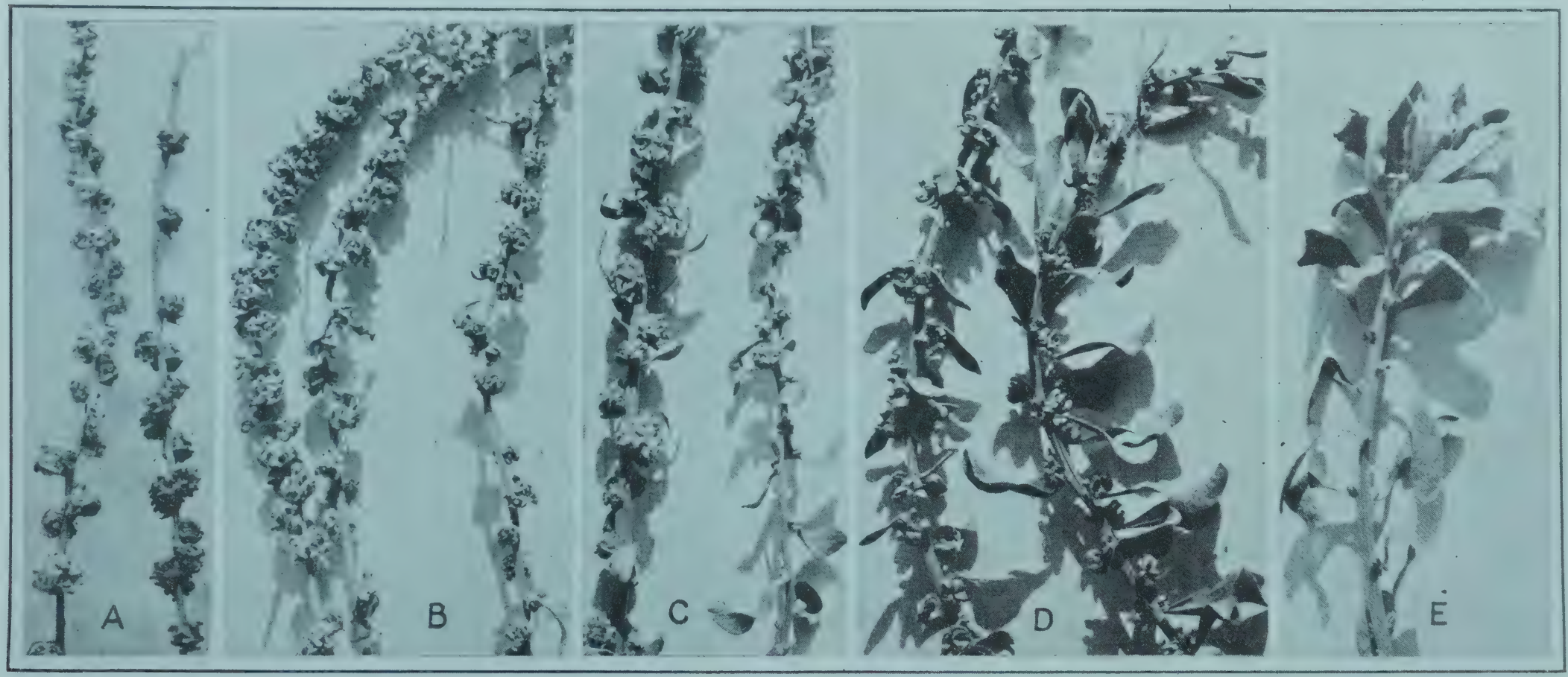
TABLE II.—*Development of seed stalks and seed, 1922–23*

Time of planting	Time required to reach final height	Time after planting beet to date of first flowering	Length of flowering period
	Days	*Days*	*Days*
Mar. 1	120	113	17
Apr. 1	90	82	19
May 1	60	52	24
June 1	45	42	25
July 1	30	30 to 60	20 to 75
Aug. 1	30	Irregular	Indefinite
Sept. 1			

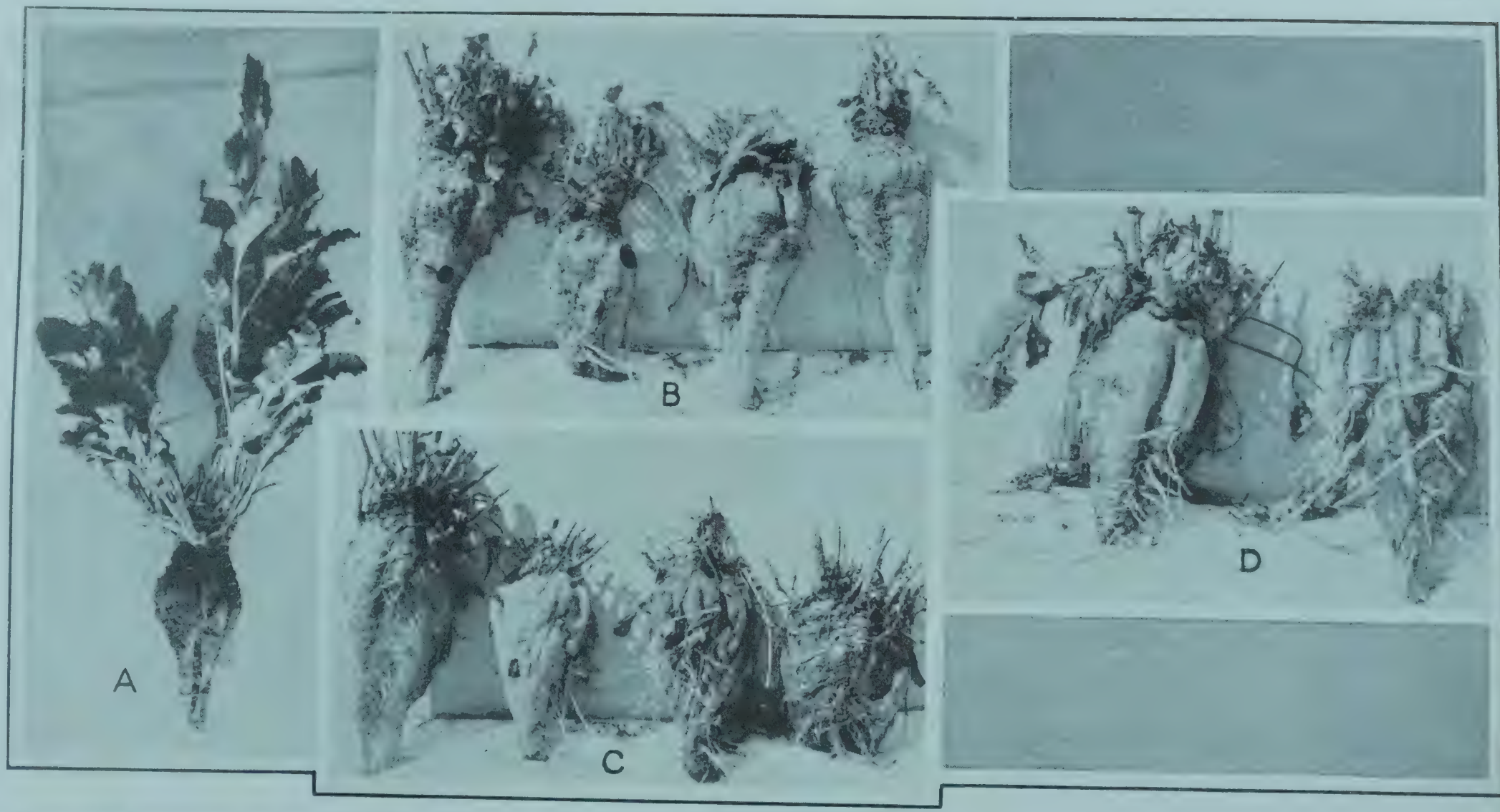
The meteorological data as to wind movement, relative humidity, and evaporation, gave no further explanation than did the effect of increased illumination. While these are all of importance, there seems to be other more important factors which require consideration. It was not until the beet roots were removed at different periods during the season that it was learned that those beets which produced the best quantity and quality of seed had previously produced the most marked root development.

Plates 2 and 3 show the root development of beets planted from April to

[3] HARRIS, F. S., and HOGENSON, J. C. SOME CORRELATIONS IN SUGAR BEETS. Genetics 1: 334–347. 1916.



A. Seed spikes from beets planted in March and April.
B. Seed spikes from beets planted in May.
C. Seed spikes from beets planted in June.
D. Seed spikes from beets planted in July and August.
E. Seed spikes from beets planted in September.



A.—Effect of additional illumination on seed stalk development

B.—Beets planted June 1

C.—Beets planted May 1

D.—Beets planted April 1

September. The root development of beets planted March 1 was similar to that of beets planted April 1. Beets planted in April and May showed some enlargement of the old root and especially a marked development of new side roots. These side roots on the beets planted early are many and robust, while they are few, very fine, and threadlike on the beets planted later in the season. In this connection it was learned from the soil and air thermograph records that the enlarged root systems were developed at a time when the soil and air temperatures were low, and that the meager threadlike root systems were developed when the soil temperature and air temperature were high. The mean air and soil temperatures for the year 1923 are given in Table III. Plate 3, C, shows extensive root development of beets planted in September, at a time when soil temperature corresponded to the soil temperature of May. It is probable that these beets would produce an abundance of seed if September and October were followed by months of high temperatures, such as June and July.

TABLE III.—*Mean air and soil (6-inch depth) temperatures for 1923*

Month	Mean air	Mean soil
	°C.	°C.
Mar.	3.9	3.0
Apr.	9.8	9.0
May	16.7	14.8
June	18.2	16.2
July	24.5	21.5
Aug.	22.6	20.7
Sept.	18.1	15.6
Oct.	11.6	9.7

In order to verify this effect of temperature more carefully, beets were planted under controlled moisture and temperature conditions ranging from 15° to 30° C. The result was that the more marked side-root development occurred between 15° and 30°. The side roots developed at higher temperatures were thin and threadlike, as shown in Plates 2, B, and 3 A, B.

In order to verify more carefully the effect of illumination on side-root development, and indirectly on seed development, beets were planted under controlled moisture and temperature conditions favorable for side-root development, and the quantity of illumination varied. Beets exposed to short or long illumination periods produced more robust and more numerous side roots than beets exposed to darkness (pl. 4, A and B). The beets planted April 1, in the field and exposed to 24-hour illumination until June 1, showed no greater root development than a similarly planted lot exposed to only 12-hour illumination. It appears that under favorable temperature conditions the usual day illumination of April and May is quite sufficient for maximum side-root development, and that the amount of illumination may not be as important as the presence or absence of illumination.

It was learned that an abundant root-bud and side-root development could be forced under controlled temperature and moisture conditions by removing crown buds (pl. 4, C). It was also possible to force crown development by removing root-bud tissues. It was further noted that temperatures of 15° and 20°C. inhibited seed-stalk development, while these same temperatures favored the development of side roots and vegetative leaves. Likewise, temperatures of 25° C. retarded the development of side roots and vegetative leaves, due to the fact that it forced seed-stalk development. Even though vegetative organs, such as leaves and side roots, may lengthen faster at higher temperatures, these same organs develop best at lower temperatures.

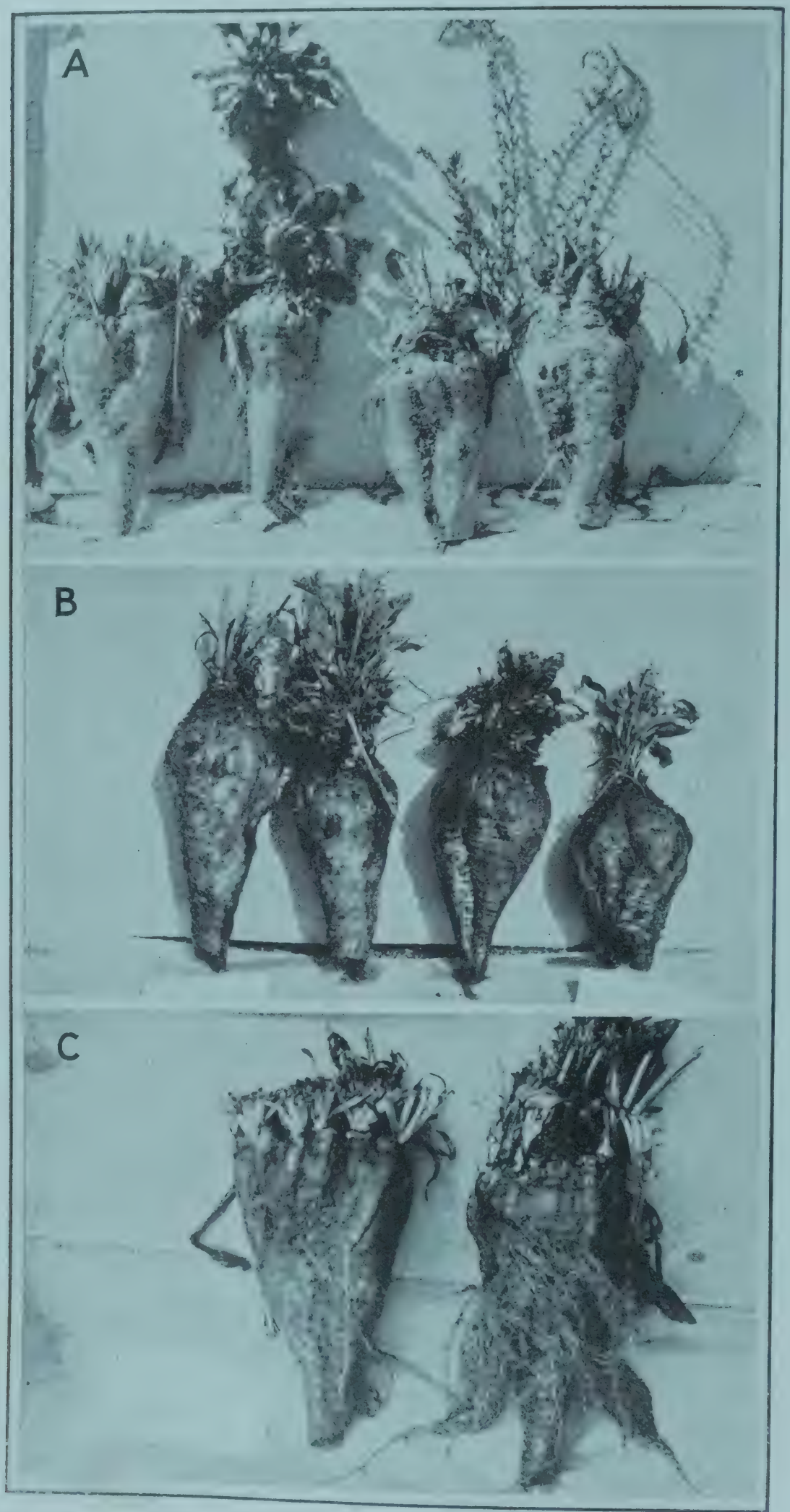
DISCUSSION

In explanation of this increased seed production resulting from early planting, Briem [4] states that the growth period of the beet from planting to blossoming time should be twice as long as the period from blossoming to ripening of seed, and that this period must have a slow, steady rise of temperature. Shaw [5] concluded from his experiments that the beets must pass through a period of restrained growth, at a temperature between 2.75° and 10° C., in order for the flower buds to develop. That this is not necessary is shown by the fact that beets stored at 1.7° or above 15° C., and planted under high temperatures in June, July, and August were not "trotzers" and produced seed.

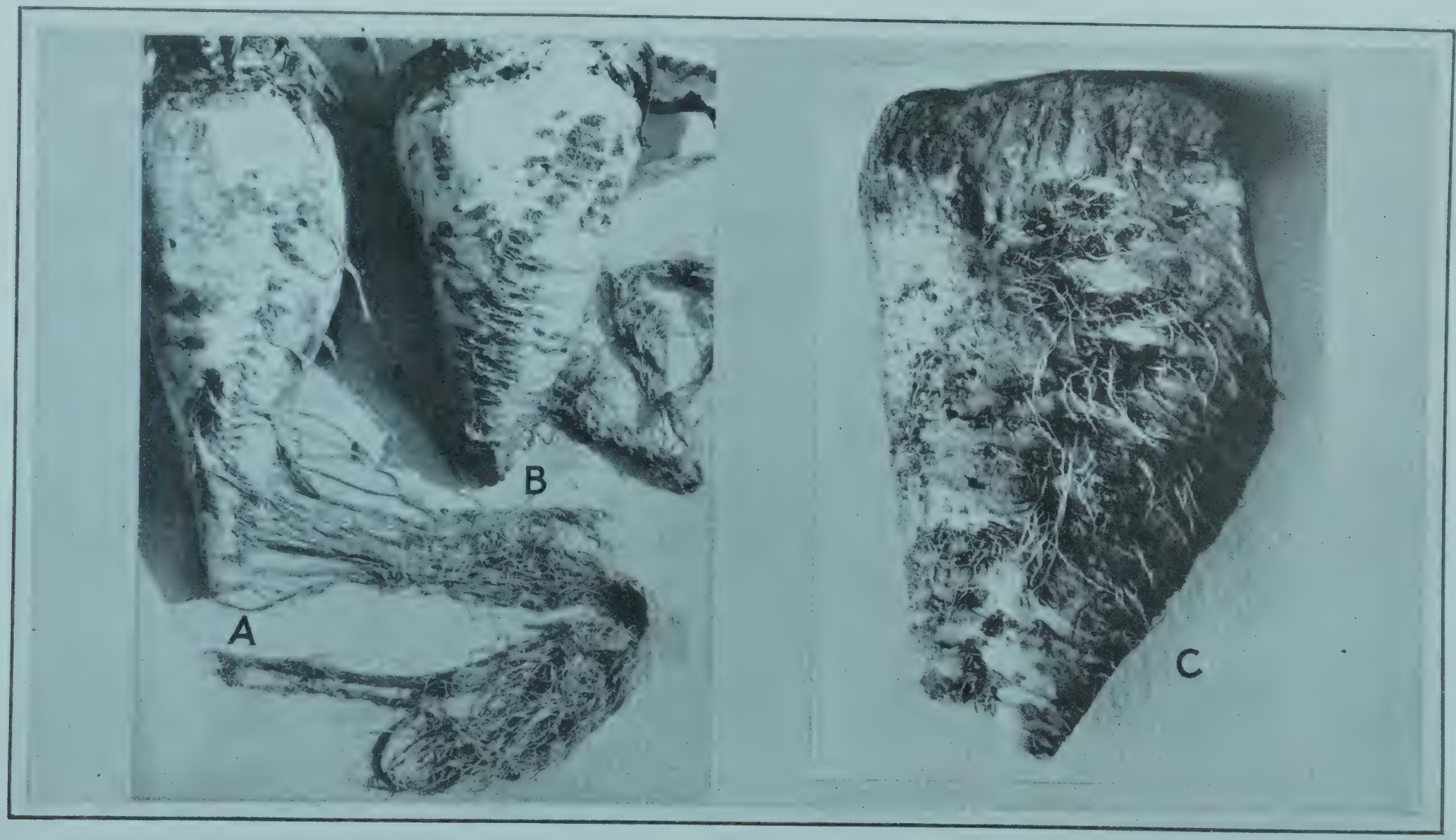
The true explanation of the relation of increased seed production with early planting of beets will probably be found in many influencing factors. It seems that certain fundamental factors, such as moisture, temperature, and aeration, must be supplied before the

[4] BRIEM, H. ZEIT DES AUSPFLANZENS UND DER SAMENERTRAG BEI MUTTERRÜBEN. Österr. Ungar. Ztschr. Zuckerindus. u. Landw. 27: 685-690. 1898.

[5] SHAW, H. B. CLIMATIC CONTROL OF THE MORPHOLOGY AND PHYSIOLOGY OF BEETS. Sugar 19: 387-391, 431-434, illus., 1917; 20: 23-27, 68-70, 109-112, 150-154, illus., 1918. 1917-18.



A.—Beets planted July 1 B.—Beets planted August 1 C.—Beets planted September 1



A.—Beets exposed to light

B.—Beets exposed to darkness

C.—Effect of removed crown on root-bud and side-root development

influence of minor factors can be considered. The best results are obtained when sound beets of good physical condition are planted early in the season. At this time the cold-air temperature inhibits seed-stalk and seed production and favors vegetative leaf production, while the cold-soil temperature is favorable for side-root production and food-mobilization processes in the beet. At this favorable soil temperature for side-root production the mother beet often shows extended enlargement with an abundant formation of new roots. This extended root system is necessary to supply water for the transpiration stream during the seed production period. In early spring the roots probably absorb limiting food materials from the soil which aid materially in the further development of the beets and especially the seed, as it is understood that the amount of available food materials in the soil varies considerably during the season. There is a further accumulation of available food materials in the beet by the digestion of the stored sucrose. These progressive changes are also accompanied by a vegetative leaf production, which undoubtedly aids in the accumulation of new food materials. As the season advances, the temperature of the soil rises to a point which is unfavorable for root production, while the temperature of the air rises to the optimum for seed-stalk and seed production. Under these conditions maximum seed production occurs, because the beet has an abundance of available food and absorption organs provided by the earlier development.

On the other hand, if beets are planted during midsummer, when the temperature of the soil is inhibitive to root growth and the temperature of the air is favorable for seed production, the beets are forced to immediate reproduction or death, and there is very little or no seed produced. In this case, all conditions are similar and favorable for maximum seed production, except that the beet has not previously passed through a period of food mobilization and vegetative development which is necessary to sustain the reproductive development.

SUMMARY

These investigations indicate that the second year's development of the mother beet should be divided into two distinct periods: The first period being characterized by food mobilization, vegetative foliage development, new root production, and absorption of soil nutrients; the second period by rapid utilization of mobilized food, development of seed stalks, and production of seed.

The optimum temperature for seed-stalk and seed development is higher than the optimum temperature for vegetative leaf and root development.

The cold air and soil temperatures during the first period inhibit the reproductive development, while these temperatures favor complete vegetative development.

The higher temperatures of the second period favor rapid reproductive development and result in the retarding of the vegetative development.

There is maximum reproduction when a plant passes through a first period of complete vegetative development in which the reproductive development is inhibited, followed by a second period of forced reproductive development in which the vegetative development is largely inhibited.

The first period is an essential prerequisite to the second.

The seed produced during the second period is directly proportional to the extent of development of the beet during the first period.

THE SOIL MULCH IN THE ABSORPTION AND RETENTION OF MOISTURE [1]

By M. A. McCall [2]

Dry Land Specialist, Washington Agricultural Experiment Station

INTRODUCTION

Without irrigation successful crop production over a large part of the western United States is dependent on moisture stored in the soil. In part, this is due to limitations in total precipitation, to its seasonal distribution, or to a combination of the two; but, in any case, the factors affecting the control of soil moisture are of the greatest practical importance.

At one time the soil mulch was assumed to be the basis of all moisture control. It was regarded as essential, both in effective absorption and in retention. More recently, however, the mulch and its necessity seem matters of doubt, and while no one advocates the discontinuance of mulch-forming tillage, former ideas at least seem to be considered untenable. In discarding former theory, however, no general principle has been developed in its stead, and so far as more than local application is concerned, the situation is somewhat hazy. As indicative of the present somewhat contradictory state of affairs, the following may be noted:

REVIEW OF LITERATURE

Barker (*4*) [3] found that at Lincoln, Nebr., the loss of water due to direct evaporation from the soil was a small factor after the water became thoroughly distributed in the soil. Young (*26*), for the same conditions, concluded that a loose soil mulch was not much more effective than an unmulched soil in retarding the evaporation of moisture already established in the soil. Burr (*8*), working at North Platte, Nebr., when starting with a soil almost filled with water, and during a season of heavy rainfall, arrived at a conclusion similar to that of Young. For a less favorable season, and with a soil comparatively dry below the second foot, Burr states that "from the viewpoint of storing water a cultivated surface is essential."

Atkinson, Buckman, and Gieseker (*3*) show that summer tillage saved moisture in excess of that retained in a soil not tilled, but no direct statement is made that the benefit of the tillage was due either to the mulch effect or to killing weeds.

Call and Sewell (*9*), after a careful study of the moisture content of a mulched and an unmulched soil, conclude that under Kansas conditions a cultivated soil is no more effective than a bare uncultivated one in preventing evaporation; and in addition conclude (*10*) that "in the past too much emphasis may have been placed on tillage as a direct means of conserving moisture and liberating plant food and too little on it for the purpose of destroying weeds." After a rather extensive review of literature from a variety of sources, Sewell (*22*) emphasizes this latter conclusion.

Merrill (*21*) quotes Farrell as reporting certain experiments at Nephi, Utah, in which fall plowing did not give beneficial results in moisture conservation; but further quotes Widtsoe as concluding from other data that "fall plowing undoubtedly conserves the winter precipitation."

Cardon (*11*) shows that at Nephi, Utah, during each of the four years 1909 to 1912, inclusive, fall-plowed soil contained less moisture than that not plowed until spring.

Harris and Jones (*16*), reporting on the same and additional data as that of Cardon, show graphically a similar result. In addition, they show that in the fall an unmulched soil contained practically as much moisture as did a mulched one. They conclude that weed removal is the most marked benefit of tillage.

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[2] The author is now Agronomist in Charge of Cereal Agronomy, Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] Reference is made by number (italic) to "Literature cited," p. 831.

Harris and Yao (*18*) state that "moisture samples taken from the Nephi substation to a depth of six feet prove in every case that fall plowing preserves more moisture than spring plowing," a statement rather difficult to understand in the light of the statements of Cardon (*11*) and Harris and Jones (*16*).

Cates and Cox (*12*), basing their conclusions on data secured in studies at widely separated points over the entire United States, conclude that weed removal is the most important function of cultivation in handling the corn crop, and that the amount of moisture saved by such cultivation is negligible beyond a possible slight checking of run-off from heavy rains.

Fortier (*13*, *14*) shows that mulching a soil after irrigation conserves moisture, and that the deeper the mulch up to 9 inches, the deepest used in his experiments, the more efficient the retention. At Wenatchee, Wash. (*14*), in a 21-day period during June, 1908, an unmulched soil lost 14.38 per cent, one with a 3-inch mulch, 3.98 per cent, one with a 6-inch mulch, 2.10 per cent, and one with a 9-inch mulch, 1.06 per cent of the water applied in a 6-inch irrigation. Willard and Humbert (*25*) show a similar result.

Harris and Bracken (*15*), from data secured under irrigation, state that cultivation is slightly more efficient in saving moisture than is no cultivation with weeds removed by pulling. They state that a comparison of deeply cultivated plats with those given shallow cultivation or no cultivation shows a decided advantage for the latter in moisture content at the lower soil depths.

McCall and Holtz (*19*) show that soils mulched during the period when moisture absorption was most active contained less moisture at the end of the fallow period than did other soils not mulched during the same absorption period. They conclude that the mulch probably aids in retaining moisture already in the soil.

Mathews (*20*), in reporting on the storage of moisture and its utilization by spring wheat, states that, as an average for all stations of the Office of Dry-Land Agriculture Investigations, Bureau of Plant Industry, United States Department of Agriculture, there are no notable differences in the moisture conserved by two continuously cropped plats, the one being fall plowed and the other spring plowed. He mentions certain seasonal conditions that may favor the first in the southern Great Plains, and the second, to the north, but that there are no consistent advantages in either case.

Shutt (*23*), in Canada, reports that a packed fallow contained more moisture than one not packed. This indicates either superior absorption or superior retention on the part of the firmly packed soil, although which is the more likely hypothesis is not suggested by the author.

From the foregoing it is clear that all investigators have not arrived at the same conclusion. It is equally clear that all are not measuring the mulch from the same point of view. In the conservation of natural precipitation the mulch must be considered both in its effect on absorption and its effect on retention. Where the mulch is measured solely as a retentive agent, as under irrigation, the evidence is unanimous that its effect is positive in saving moisture. In measuring the value of the mulch in relation to natural precipitation, where both considerations come into play, the result is less clear and the advantages, if any, are slight, neutral, or negative.

INVESTIGATIONS

Soil-moisture problems are a very important part of the program of the Adams Branch Station of the Washington Agricultural Experiment Station at Lind, Wash. Production in the territory this station serves is based on the summer-fallow system of alternate years of crop and fallow. One of the objects of the fallow is to conserve moisture and to carry it from one season to the next. As one of the factors affecting moisture conservation, the soil mulch has been studied during the six years 1918 to 1923, inclusive.

Under field conditions it is often difficult to differentiate between the two different relationships of the mulch. This is particularly true when the period of greatest precipitation is concurrent with that of greatest evaporation, as, for instance, in the Great Plains area. Climatic conditions on the Adams Station, however, meet the requirements for differentiation to a considerable degree. The distribution of rainfall, and the seasonal fluctuation of temperature and relative humidity (figs. 1, 2, and 3), divide the year into distinct periods, during any one of which either absorption or retention becomes most prominent. During the late fall, winter, and early spring, when a good proportion of all precipitation occurs, relative humidity is comparatively high and absorption is the most important consideration. During the summer temperatures are

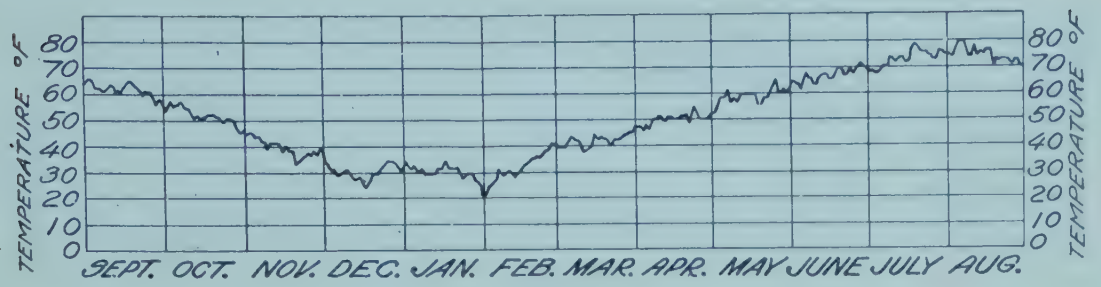

FIG. 1.—Mean daily temperature, based on an 11-year average, at Lind, Wash. During the period from November 15 to March 15, when mean temperatures are comparatively low, evaporation is less active than at any other time, and the opportunity for precipitation to be absorbed by the soil is correspondingly greater. The general relationship between mean temperature and rate of evaporation is shown in Figure 2

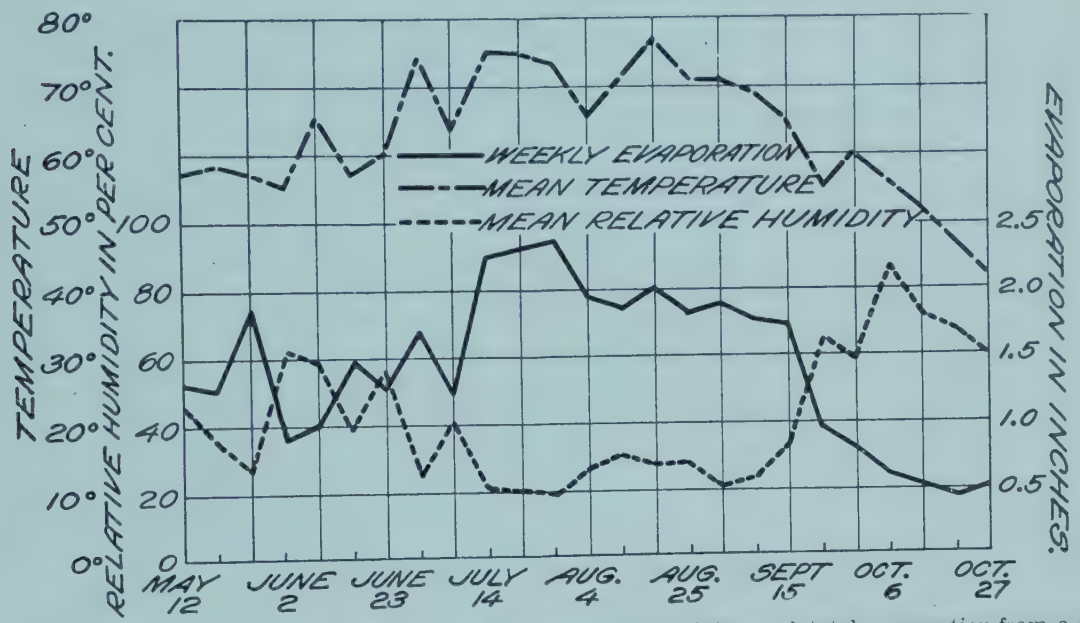

FIG. 2.—Relation between mean temperature, mean relative humidity, and total evaporation from a free water surface, by weeks, at Lind, Wash., May 9 to October 30, 1924. The dates given represent weekly centers

high, relative humidity is low, and little or no rainfall occurs. Retention then becomes paramount. The fallow period, from a moisture standpoint, extends from the removal of one crop to the beginning of the growing season of the next. Practically, on the Adams Station, it therefore consists of a first period of absorption, extending through the first fall, winter, and spring, followed during the summer by a period in which retention is important, and this, in turn, followed by a second period of absorption. The soil moisture content at the beginning and end of either of these periods should, therefore, give a basis for reliably measuring the effect of the mulch in whichever function is most important for that period.

The soil of the Adams Station is a very fine sandy loam of the Ritzville series. It is a typical semiarid soil, deep and uniform. The mechanical analysis of the soil by feet to a depth of 4 feet is given in Table I.

TABLE I.—*Mechanical analysis, by feet, to a depth of 4 feet of the soil on the Adams Branch Station, Lind, Wash. (19).*

Soil section	Fine gravel	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt and clay	Total
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
First foot	0.00	0.08	0.13	9.82	45.88	45.05	100.96
Second foot	.00	.08	.08	8.68	46.75	44.40	99.99
Third foot	.00	.11	.14	8.04	52.01	39.70	100.00
Fourth foot	.00	.06	.05	6.10	50.24	43.45	99.90

The study of the mulch was made on the plats of the summer-fallow experiments. There are two series of these plats, one series being in crop and one in fallow each year. The plats are one-twentieth of an acre in area, 1 rod wide by 8 rods long.

Samples for moisture determinations were taken by foot sections to a depth of 4 feet, this depth being sufficient during the period of somewhat deficient moisture covered by the study.

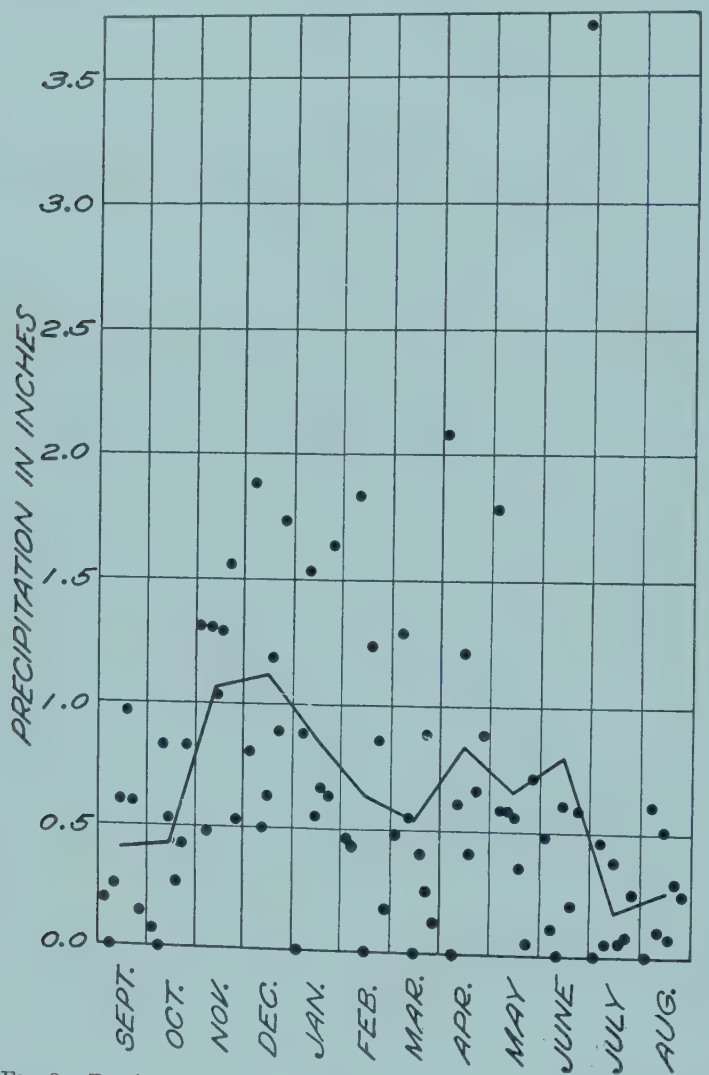

FIG. 3.—Precipitation by months, at Lind, Wash., September 1, 1916, to August 31, 1923. The tendency for greater precipitation during the winter months is apparent, as well as the unusual rainfall of June, 1923

Methods of sampling and of determining soil moisture are given in Bulletin 164 of the Washington Agricultural Experiment Station (*19*).

Moisture data are given in acre-inches, rather than in per cent of moisture-free soil. The volume weight of the surface 6 inches of a plowed soil is materially different from that of the same soil in an undisturbed condition, and the same per cent moisture content does not signify the same amount of moisture present in the same effective depth in the two cases. In the soil on which this study was made the volume weight of the plowed layer is approximately 60 per cent of that before stirring. The volume weight of the surface foot of such a plowed soil is 80 per cent that of the unstirred. Moisture content of the surface foot, where necessary, was calculated on this basis.

In Table II are given total soil moisture data for certain tillage variations in preparing the fallow, which indicate the reaction of the mulch in absorption each year during the period 1918 to 1923, inclusive.

All of the tillage variations as given in Table II were maintained in a well-mulched condition during the summer period of retention. So far as this period and the succeeding period are concerned, the result for all should have been similar. The essential difference between the variations was in the presence or absence of a soil mulch during all or a part of the first period of absorption. The only plats on which no mulch existed at any time during this first period were those of the early spring plowing with no previous treatment. All of the others were mulched for varying periods, either with a light mulch created by disking in August immediately after harvesting the preceding crop, or with a heavier mulch created by plowing at some later date.

Not once during the 6-year period did any of the mulched plats equal the unmulched plats in final moisture content. In several instances the shallow mulch of the harvest-disked and spring-plowed plats was almost as efficient in saving moisture, but this efficiency was not due to equality in moisture absorption. This is shown by comparing the dry fall-plowing alone and the dry fall-plowing with previous harvest-disking. As far as absorption was concerned, these two treatments should have given an identical effect, the difference between the two being in the presence or absence of weed growth in the stubble in the interim between harvest and the date of dry fall plowing. The comparative result between these two treatments is not always the same, due to the fact that weeds were not always present in the stubble, but the

TABLE II.—*A comparison of total and conserved soil moisture in the soil to a depth of 4 feet after the year of fallow, for six variations in fallow preparation*

[The essential difference between these variations lies in the presence or absence of a soil mulch during all or a part of the first major period of moisture absorption. Adams Branch Station, Lind, Wash., 1918 to 1923, inclusive]

Tillage variations	Soil moisture in acre-inches to a depth of 4 feet after the year of fallow								
	1918	1919	1920	1921	1922	1923	Average	Conserved moisture [a]	Comparative amount of conserved moisture
Dry fall-plowed	3.35	4.97	4.89	5.07	4.67	5.00	4.66	2.58	100
Harvest-disked, dry fall-plowed	4.34	5.34	4.76	4.82	5.48	5.03	4.96	2.88	111
Harvest-disked, wet fall-plowed	3.94	5.54	4.58	5.05	5.24	4.76	4.85	2.77	107
Harvest-disked, early spring-plowed	4.61	5.63	4.87	5.52	6.18	5.52	5.39	3.31	128
Wet fall-plowed	3.75	5.49	4.80	5.04	5.18	5.57	4.97	2.89	112
Early spring-plowed	4.70	5.98	5.32	5.76	6.47	5.60	5.64	3.56	138

[a] Residual moisture, 2.08 acre-inches to a depth of 4 feet, average of four seasons.

data indicate that on the average there was some saving of moisture through weed removal by harvest-disking. Despite this saving, however, the effect on absorption more than counterbalanced the gain from killing weeds, as shown by the slightly lower average moisture content of the harvest disking and early spring plowing, when compared with spring plowing with no previous treatment. A comparison of harvest-disking and wet fall-plowing with wet fall-plowing alone shows the same effect, due to the same cause.

It might be suggested that the moisture advantage of the spring-plowed soil was due entirely to the holding of snow by stubble. This factor undoubtedly is of some importance under certain conditions, but, so far as this experiment was concerned, it was relatively unimportant in determining total moisture. The effect of the mulch on absorption played a much larger part in the final result. This is indicated by comparing the moisture content of the harvest-disking and spring-plowing with that of the harvest-disking and dry fall-plowing. Stubble was not extensive on any of the plats, and as a result of the tillage, neither of these variations carried a stubble cover during the period of snowfall. The lower average moisture content of the second must be attributed, therefore, to a more pronounced inhibitory effect due to the deeper mulch of the dry plowing as compared with that of the disking. This indicates that, within certain limits, the inhibitory effect is proportional to depth in the mulch. That the effect of the mulch on absorption is not due to the physical condition resulting from tilling this particular soil when dry is shown by the fact that the mulch created by the wet fall-plowing also reacted in inhibiting absorption.

It is apparent, therefore, that during each of the six fallow periods covered by these data, soil covered by a mulch during all or a part of the first period of absorption contained less moisture at the end of the fallow than where no mulch existed during the same period of absorption. This indicates that the lower moisture content was due to an inhibiting effect of the mulch on absorption.

ABSORPTION

Detailed data on absorption are available for the seasons of 1920 to 1923, inclusive, and on retention for the seasons of 1921 to 1923, inclusive. In the study of absorption certain plats of the experiments, mulched and unmulched, were sampled at the beginning and at the end of each major absorption period. In the study of retention two plats were given no surface tillage whatever, but were kept free of growth by shaving with a sharp hoe. Any loose soil was removed by winds, and the surface of these plats was as free from both weeds and mulch as could be desired. The moisture content of these plats at the beginning and the end of the retention period was compared with that of other plats mulched during the same period. The data on absorption (Table III) will now be considered, followed by the results on retention (Table IV).

In the spring of 1921 the unmulched soil had absorbed 3.53 inches of water per acre. This was approximately 67 per cent of the total precipitation

TABLE III.—*Soil-moisture content in acre-inches by foot sections to a depth of 4 feet in mulched and unmulched soils, at the beginning and at the end of the first absorption period of the fallow, together with gains in moisture during the absorption period, Adams Branch Station, Lind, Wash., 1920 to 1923, inclusive*

Soil section	1920–1921						1921–1922					
	Mulched, fall plowed			Unmulched, stubble			Mulched, fall disked			Unmulched, stubble		
	Aug. 16, 1920	Apr. 14, 1921	Gain	Aug. 16, 1920	Apr. 14, 1921	Gain	Aug. 10, 1921	Apr. 7, 1922	Gain	Aug. 10, 1921	Apr. 7, 1922	Gain
First foot	0.24	1.52	1.28	0.24	1.76	1.52	0.28	1.70	1.42	0.27	2.09	1.82
Second foot	.49	1.65	1.16	.49	1.66	1.17	.56	1.42	.86	.52	1.59	1.07
Third foot	.56	1.14	.58	.57	1.32	.75	.64	.69	.05	.63	.75	.12
Fourth foot	.57	.61	.04	.57	.66	.09	.60	.65	.05	.63	.72	.09
Total gain			3.06			3.53			2.38			3.10
Gain below first foot			1.78			2.01			.96			1.28
Rainfall during interval, in inches			5.26			5.26			5.30			5.30

Soil section	1922–1923						3-year average					
	Mulched, fall plowed			Unmulched, stubble			Mulched			Unmulched		
	Sept. 7, 1922	Mar. 23, 1923	Gain	Sept. 7, 1922	Mar. 23, 1923	Gain	Fall	Spring	Gain	Fall	Spring	Gain
First foot	0.26	1.21	0.95	0.25	1.63	1.38	0.26	1.48	1.22	0.25	1.83	1.58
Second foot	.54	1.15	.61	.52	1.57	1.05	.53	1.41	.88	.51	1.61	1.10
Third foot	.61	.60	.01	.64	1.04	.40	.60	.81	.21	.61	1.04	.43
Fourth foot	.57	.60	.03	.63	.49	.14	.58	.62	.04	.61	.62	.01
Total gain			1.58			2.69			2.35			3.12
Gain below first foot			.63			1.31			1.13			1.54
Rainfall during interval, in inches			5.08			5.08			5.21			5.21

that had fallen between the dates of sampling. The mulched soil had absorbed 3.06 inches during the same period, or 58 per cent of the total precipitation. Depth of absorption is an important factor in subsequent retention. In the unmulched soil 2.01 inches of moisture had penetrated below the first foot, while in the mulched soil 1.78 inches had reached this same lower level. The unmulched soil contained 12 per cent more moisture in the area where it could be most effectively retained.

In the spring of 1922 the soil not mulched during the preceding winter had absorbed 3.10 inches of moisture, and of this amount 1.28 inches was below the first foot. The mulched soil in the same time had absorbed 2.38 inches, with 0.96 inch below a depth of 1 foot. During this period the unmulched and mulched soils absorbed and retained, respectively, 58 per cent and 45 per cent of the total precipitation. In total absorption, the unmulched soil was 30 per cent superior to the mulched, and in the amount penetrating below the first foot was 22 per cent superior.

In the spring of 1923 the data show that the unmulched soil had absorbed 2.69 inches of moisture in excess of that present the previous fall. Of this amount, 1.31 inches was below the first foot. The mulched soil absorbed 1.58 inches, with only 0.63 inch at the lower levels. The data for the spring sampling of the unmulched soil indicate that a loss occurred in the fourth foot of this treatment after soil moisture was determined the previous fall. This loss was due to weed growth (Russian thistle, *Salsola kali tenuifolia* G. F. W. Mey) subsequent to the date of dry fall-plowing, and after the moisture samplings were made. Despite this moisture loss from the unmulched soil above that occurring from the mulched treatment, absorption was sufficiently greater where the mulch was absent to entirely recover this loss and with substantial additions in all sections except in the fourth foot. Total absorption in the unmulched

soil was actually the absorption shown by the data, plus the undetermined loss. On the basis of the data, the unmulched soil absorbed 53 per cent and the mulched soil absorbed 31 per cent of the total intervening precipitation. The unmulched soil was 69 per cent superior in total absorption and slightly over 200 per cent superior in the amount of moisture below the first foot.

In every instance the unmulched soil was superior as an absorbing agent. The comparative efficiency of the mulched and unmulched soils in absorption is shown graphically in Figure 4.

In 1922 the loss from the mulched soil amounted to 0.71 inch, and, considering the gain in the lower 2 feet, this loss came entirely from the first foot. The unmulched soil sustained a loss of 1.61 inches, and of this loss 0.21 inch came from below the first foot. Neither the mulched nor the unmulched soil lost moisture from the third foot in 1922.

In 1923 the mulched soil showed a total loss from July 6 to September 20 of 0.49 inch. Of the total loss, but 0.04 inch came from below the first foot, and none was lost from the third foot by evaporation. The unmulched soil in the same period lost 0.95 inch

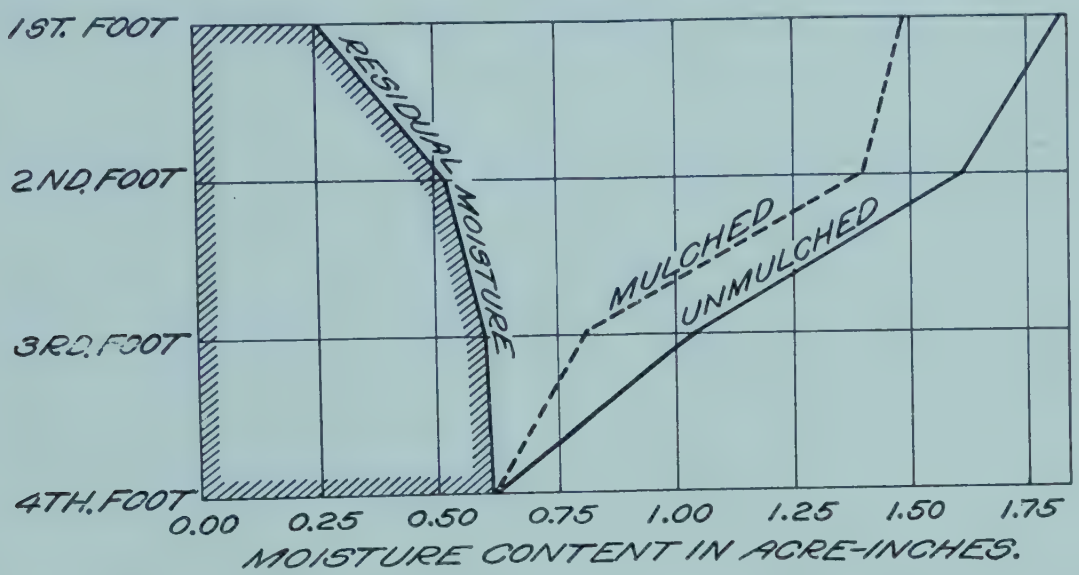

FIG. 4.—Average moisture content in acre-inches of mulched and unmulched soils at the end of the first period of moisture absorption during the fallow year, Adams Branch Station, Lind, Wash., 1920 to 1923, inclusive

RETENTION

Data showing the relation of the mulch to retention are given in Table IV. These data show that during the summer of 1921 the soil covered by a 6-inch soil mulch lost 1.02 inches of moisture. Of this total loss by evaporation, 0.12 inch came from below the first foot. In the lower 2 feet of this soil there was a gain by downward movement of 0.27 inch. The soil covered by a 4-inch mulch lost a total of 1.59 inches of moisture, or 56 per cent more than the 6-inch mulched soil. Of this amount 0.55 inch came from below the first foot, and 0.10 inch came from the third foot. The unmulched soil lost 2.02 inches of moisture. This was 198 per cent of that lost from the 6-inch mulch. Of the total lost from the unmulched soil, 0.85 inch came from below the first foot, and 0.32 inch came from the third foot.

of moisture, with 0.32 inch coming from below the first foot. A very small loss by evaporation was indicated from the third foot of the unmulched soil.

The data for retention show the mulch to have a positive effect in conserving moisture, when considered only from that standpoint, and that efficiency in retention is increased by depth in the mulch. The comparative efficiency of the mulch in retention is shown in Figure 5.

CONCURRENT ABSORPTION AND RETENTION

Ordinarily, on the Adams Station, the mulch need be considered only as a retentive agent during the summer season, but in June, 1923, a rainfall of 3.69 inches gave an opportunity to observe it as an absorbing agent under such conditions also. In effect, the mulch was functioning in absorption and retention at one and the same time (Table V).

TABLE IV.—*The soil-moisture content in acre-inches by foot sections to a depth of 4 feet, in mulched and unmulched soils, at the beginning and at the end of the period of extreme summer evaporation during the year of fallow, together with moisture losses occurring during the period, Adams Branch Station, Lind, Wash., 1921 to 1923, inclusive*

Soil section	1921									1922					
	6-inch mulch			4-inch mulch			Unmulched			Mulched			Unmulched		
	Apr. 14	Sept. 3	Loss	Apr. 14	Sept. 3	Loss	Apr. 14	Sept. 3	Loss	Apr. 7	Sept. 6	Loss	Apr. 7	Sept. 6	Loss
First foot	1.63	0.73	0.90	1.76	0.72	1.04	1.66	0.49	1.17	1.70	0.81	0.89	2.09	0.69	1.40
Second foot	1.65	1.26	.39	1.66	1.21	.45	1.56	1.03	.53	1.42	1.28	.14	1.59	1.15	.44
Third foot	1.18	1.18	.00	1.32	1.08	.24	1.41	.98	.43	.69	.96	[a].27	.75	.95	[a].20
Fourth foot	.62	.89	[a].27	.66	.80	[a].14	.61	.72	[a].11	.65	.70	[a].05	.72	.75	[a].03
Total loss			1.02			1.59			2.02			.71			1.61
Loss below first foot			.12			.55			.85			.18			.21
Loss from third foot by upward movement			.00			.10			.32			.00			.00
Gain in fourth foot			[a].27			[a].14			[a].11			[a].05			[a].03

Soil section	1923						3-year average					
	Mulched			Unmulched			Mulched			Unmulched		
	July 6	Sept. 20	Loss.	July 6	Sept. 20	Loss	Spring	Fall	Loss	Spring	Fall	Loss
First foot	1.34	0.89	0.45	1.42	0.79	0.63	1.56	0.81	0.75	1.72	0.66	1.06
Second foot	1.74	1.39	.35	1.51	1.21	.30	1.60	1.31	.29	1.55	1.13	.42
Third foot	1.41	1.33	.08	1.29	1.17	.12	1.99	1.16	[a].07	1.15	1.03	.12
Fourth foot	.72	1.11	[a].39	1.11	1.21	[a].10	.66	.90	[a].24	.81	.89	[a].08
Total loss			.49			.95			.73			1.52
Loss below first foot			.04			.32			.02			.46
Loss from third foot by upward movement			.00			.02			.00			.04
Gain in fourth foot			[a].39			[a].10			[a].24			[a].08

[a] Gain.

TABLE V.—*Soil moisture content in acre-inches to a depth of 4 feet of a mulched and an unmulched soil at the beginning and at the end of a period of summer precipitation, Adams Branch Station, Lind, Wash., 1923*

Soil section	Mulched			Unmulched		
	May 31	July 6	Gain	May 31	July 6	Gain
First foot	1.03	1.34	0.31	1.48	1.42	−0.06
Second foot	1.41	1.74	0.33	1.32	1.51	0.19
Third foot	1.12	1.41	0.29	1.15	1.29	0.14
Fourth foot	0.59	0.72	0.13	1.01	1.11	0.10
Total gain			1.06			0.37
Gain below first foot			0.75			0.43
Rainfall during interval, in inches			3.69			3.69

Of the total rainfall of 3.69 inches occurring between May 31 and July 6, 1923, the mulched soil absorbed 1.06 inches. This was 28.7 per cent of the entire amount. Of the total amount absorbed, reference to Table IV shows that practically one-half was lost by evaporation during the remainder of the summer, as well as such limited additional rain as fell in the interim. By September 20 only 15.4 per cent of the entire June rainfall remained in the mulched soil. The unmulched soil had absorbed and retained only 0.37 inch of the June rainfall on July 6, or 10 per cent of the total. By September 20, even this small amount had been lost, as well as an additional 0.58 inch from that previously in the soil.

In a general way, the conditions prevailing during this period and the final result were both very similar to those characteristic of the Great Plains area, and their proper interpretation should give a basis for understanding the somewhat divergent data from this region and that west of the Rocky Mountains.

DISCUSSION

In most instances data have not been reported by other investigators in a form lending itself to a true evaluation of the mulch. The data from the Nephi (Utah) Substation, as reported by Cardon (*11*) and by Harris and Jones (*16*), the two being in substantial agreement, do, however, lend themselves to a measurement of the absorptive effect. Data after Cardon are shown graphically in Figure 6.

Regarding these data, Cardon (*11*) says:

"The facts thus brought out seem to indicate that at Nephi stubble land allows the winter precipitation to penetrate to greater depths than fall-

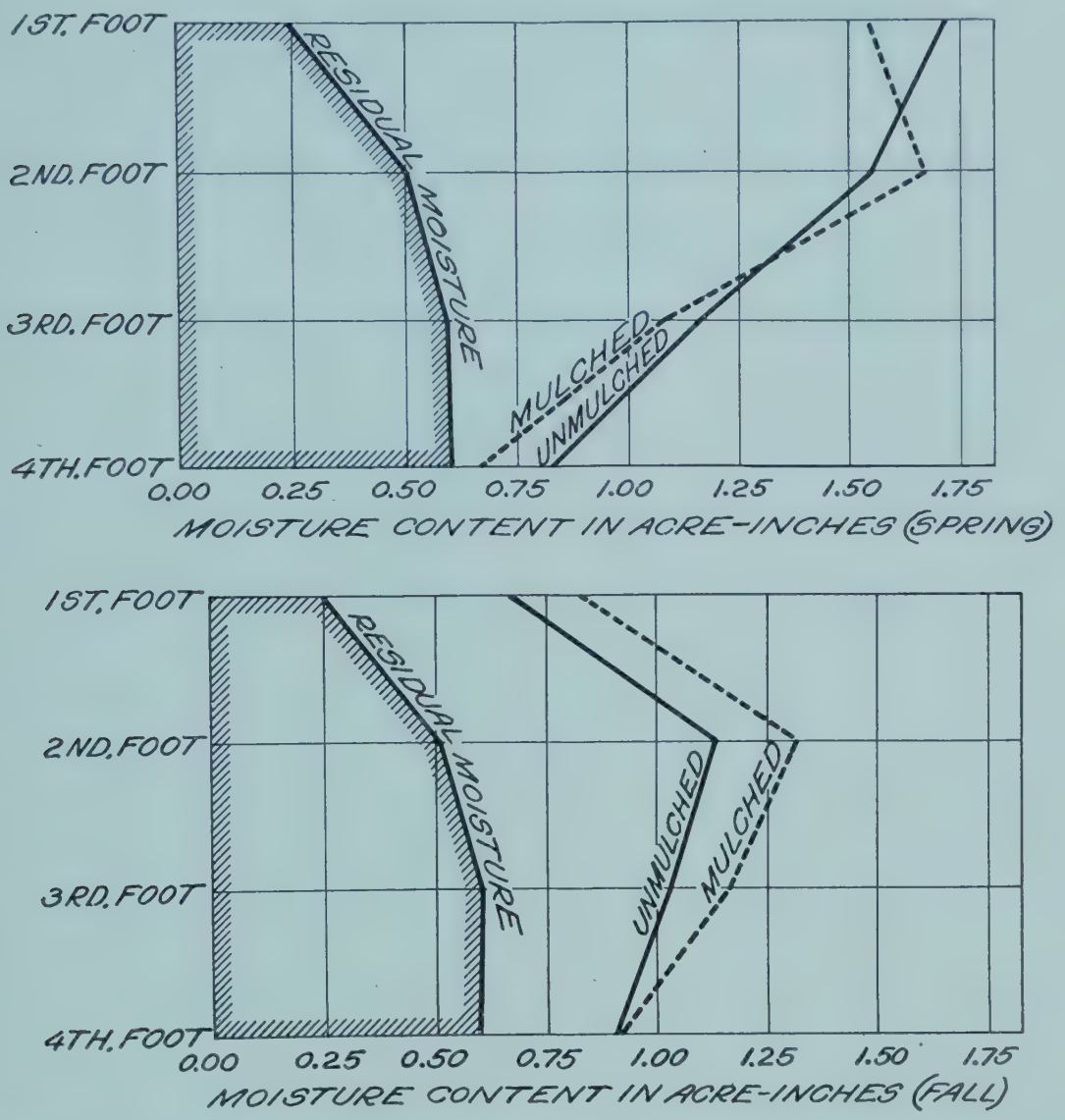

FIG. 5.—Average moisture content in acre-inches of mulched and unmulched soils in the spring and in the fall of the fallow year, Adams Branch Station, Lind, Wash., 1920 to 1923, inclusive

plowed land, and that the loose surface of the fall-plowed land retains more of the precipitation of winter than the compact surface of the stubble land."

Cardon recognized that fall plowing hindered moisture absorption, although he did not directly attribute the effect to the mulch as an inhibiting agent.

The various data presented having shown the mulch to be an inhibitory agent in absorption and a positive agent in retention, the fundamental reasons for these reactions merit attention.

On the Adams Station the prevailing type of rainfall is characteristically in the form of light, intermittent showers. Very rarely does a single rain total 0.50 inch, and the majority of individual showers amount to 0.30 inch or less. Cardon (*11*) says:

"Most of the rainstorms at Nephi have been small and generally almost negligible." This type of rainfall undoubtedly is a factor in the inhibitory reaction of the mulch.

When sampling, the surface 6 inches of a mulched and an unmulched soil immediately after a rain, the moisture content of the mulched soil on a percentage basis is always greater, the loose soil having more moisture in proportion to soil in the surface area. This fact was noted by Cardon (*11*) and by Harris and Jones (*16*).

Widtsoe and McLaughlin (*24*) and Fortier (*13*) have called attention to the fact that evaporation is always greater from a soil the greater the percentage of moisture present, or, as stated by the former, "the rate of loss of water from a soil increases as the initial per cent of water in the soil increases." This means that drying winds or other factors favoring evaporation immediately following a rain will more quickly remove moisture from the loose soil of a mulched surface than from the more firm soil in an undisturbed condition. After an intervening period of evaporation, a second shower falling on the two soils, because of the larger amount of moisture remaining in the unstirred soil, is absorbed and conducted more deeply into the unstirred soil than where the mulch is present. Alway and McDole (*2*) have shown that depth of penetration increases in direct proportion to initial moisture content, as has also been noted by Burr (*8*) and by Buckingham (*7*). Such deeper penetration, following a series of showers with intervening periods of evaporation, gives the unstirred soil its final moisture advantage, the result being due to the cumulative effect of intermittent rainfall and intervening evaporation, rather than to original superior penetration in the more firm soil. A light, intermittent rainfall contributes to the result, since heavy showers, by wetting entirely through the mulch, destroy the mulch effect, the mulch in such cases being an actual benefit in so far as it prevents surface run-off.

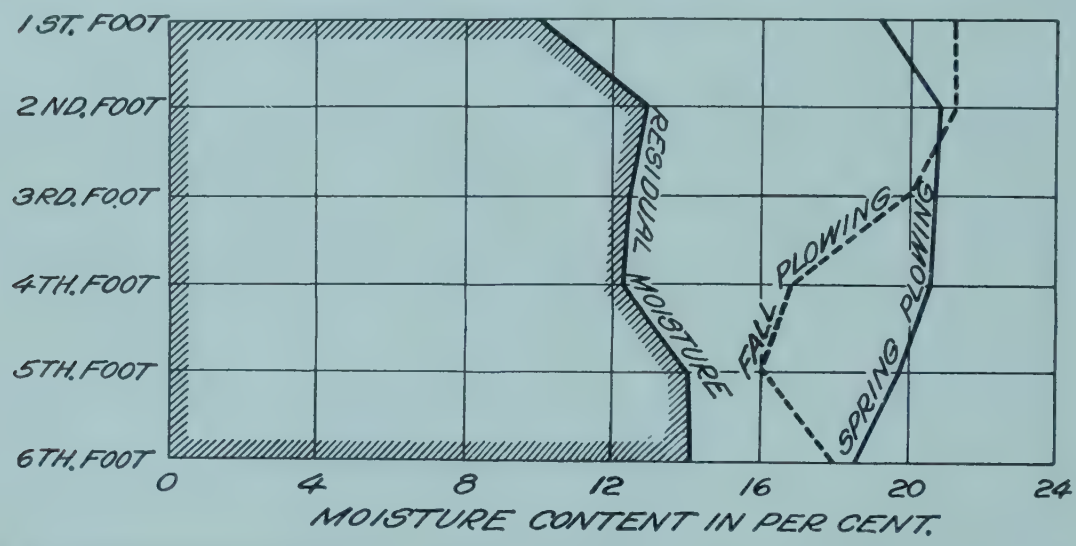

FIG. 6.—Average moisture content in per cent of moisture-free soil of fall-plowed and spring-plowed soils at the end of the first winter of the fallow, Nephi, Utah, 1909 to 1912, inclusive

Bouyoucos (*6*) has shown that when freezing occurs in soils not saturated, there is a withdrawal of soil water from the finer capillaries into the larger pore spaces. Dry-farm soils are seldom so saturated that the familiar heaving effect of more humid regions occurs, but it is possible that there might occur in freezing a certain withdrawal of moisture from the more firm soil below into the larger interspaces of a loose mulch. With subsequent thawing, a larger proportion of moisture would thus be left

near the surface exposed to evaporation. This factor might have a bearing on increasing the inhibitory effect of the mulch on absorption during the colder seasons of the year, but if so it has been less important on the Adams Station than the other factors outlined above, as evidenced by the fact that the inhibitory effect has been apparent before any freezing weather occurred.

That the inhibitory effect of the mulch on absorption tends to be proportional to the depth of the mulch was brought out by discussion of the data in Table II. The deeper mulch of the dry fall plowing was a more effective inhibiting agent than the more shallow mulch of the disking. Any condition which tends to retain the largest percentage of current showers near the surface undoubtedly would aggravate the inhibitory effect of the mulch on absorption, and increased depth in the mulch has such an effect. Under normal field conditions a stirred soil tends naturally to settle as it is wet, and a deeper mulch, requiring a larger amount of moisture to entirely wet through it, will naturally remain an effective inhibiting agent for a longer time than would one more shallow. The volume and frequency of individual rains naturally have a very important bearing on this factor.

In no instance has the writer been able to find a record of the mulch failing to conserve moisture when it is clearly shown that it was confined to that single function. The data presented in this study for the summer periods are, therefore, in complete agreement with the findings of others so far as this one phase is concerned.

In general, the theoretical operation of the soil mulch in preventing the loss of soil moisture through evaporation might seem too well known to require an extended discussion; yet, because the issue has been confused in some cases, a certain elaboration is desirable. It has generally been considered as established beyond controversy that soil moisture moves within the soil in response to the forces of capillarity. In accordance with this concept, as evaporation removes moisture from the surface soil, other moisture moves up from more moist underlying soil, and being in turn removed by evaporation, a more or less continuous movement from below to the surface is established. The stirring, incident to creating the soil mulch, decreases the number of points of capillary contact, and, by exposing additional surface to evaporating agencies, increases evaporation in the immediate surface soil to a rate beyond the ability of capillary movement to replace losses. As a result, the surface soil becomes dry, and being already loosened by the stirring, the mulch effect is created. Buckingham (*7*), Alway and Clark (*1*), and others have shown that such a dry soil retards capillary movement, hence, when present as a loose surface layer, necessarily would check evaporation.

In introducing the review of literature in their discussion of the soil mulch, Call and Sewell (*9*) make the statement that "capillary movement without the presence of a water table (with a dry subsoil) has not been demonstrated." From the data presented in Table IV, it is very evident that moisture withdrawal, and hence movement, occurred in both the mulched and unmulched soils. This movement was both upward and downward, but despite the fact that downward movement, aided by gravity, tends to be greater than that in an upward direction, as noted by Harris and Turpin (*17*), this latter was slight in the unmulched soil, and, varying with season, there occurred a withdrawal of moisture extending into the third foot. How much deeper this upward movement might or might not have extended with greater moisture content and deeper penetration is, of course, a matter of conjecture, yet it was extensive enough in any case to demonstrate that upward movement occurs without a water table. That this was a capillary movement seems the logical conclusion, since Buckingham (*7*) and Bouyoucos (*5*) both have shown that internal evaporation and diffusion have very little effect on moisture movement within the soil.

It is well known that the rapid evaporation of arid regions often greatly exceeds the ability of any soil to conduct moisture to the surface by capillarity, resulting in a naturally dry surface condition, this dry surface acting in preventing further loss of moisture as a "natural mulch" (*7*). Many have felt that such a natural mulch might make unnecessary the artificially created one, and that benefits have been ascribed to the latter which may, in reality, be just as effectively secured by natural means and without unnecessary labor. The data presented, however, would not indicate the "natural mulch" to be equal to the artificially created one on the Adams Station, and, eliminating concurrent precipitation and its effects on soil moisture content in the mulched and unmulched soil, it seems very doubtful

if the "natural mulch" is ever equal in efficiency to the artificial in the actual retention of moisture already in the soil.

Since there is, therefore, a proved upward movement of moisture in the soil under conditions of rapid removal by evaporation, and since the data presented show that the soil mulch can and does check a certain part of this movement and loss, there can be little doubt that as a purely retentive agent, the soil mulch has a positive effect.

The two effects of the soil mulch in absorption and retention have been shown and their operation has been described. The data for the period of summer rainfall indicate how these two effects may be modified by type of rainfall and intensity of evaporation. In the particular June period considered in this study there were two individual rains, each greater than 1 inch in amount, and both of which penetrated well below the mulch. Following these rains evaporation was rather active. When volume of rainfall is great enough to penetrate the mulch and to establish connection with the underlying soil, the mulch no longer acts in absorption in the same manner as with lighter precipitation. If conditions favor rather intense evaporation, tillage given immediately after such rainfall, by renewing the mulch, may retain a certain part of the moisture which has penetrated below the mulched area. When the mulch is not reestablished after the rain, under such conditions there is greater loss of moisture from the unmulched soil. In conserving natural precipitation, the most important consideration is to reduce evaporation in the surface area to a point that moisture may have an opportunity to move downward into the lower soil. If the rate of evaporation is not extreme, and moisture falling on the surface is not removed, either as run-off or by evaporation, before it has an opportunity to penetrate to lower levels, there is nothing to be gained by creating a mulch, either before or after a period of rainfall. Light showers not sufficient to penetrate the mulch have little effect one way or the other on total moisture content, for either in a mulched or an unmulched soil such a limited amount of moisture near the surface is quickly removed if evaporation be active. The value of the soil mulch in conserving moisture in a region of summer rainfall, therefore, is entirely dependent on the volume of individual showers and on the intensity of succeeding evaporation. Practically this may result in little effect from the mulch one way or the other, as shown, for instance, by the data reported by Call and Sewell (*9*), by Barker (*4*), by Young (*26*), and others.

In the foregoing discussion there is no intent to convey the idea that the balance of the inhibitory effect of the mulch on absorption and its positive effect in retention is the sole determiner of the final result in tillage practice. Soil type, temperature, relative humidity, the volume, character and distribution of precipitation, etc., may give other factors prominence so that the presence or absence of the mulch is not always of equal importance. In districts of heavy snowfall and of comparatively high winds the protective effect of a heavy stubble in holding snow or in checking the evaporative influence of the wind may contribute very largely, for instance, to final soil moisture content; and in such a case all of the benefits can not be ascribed to the absence of a mulch that might hinder absorption. If precipitation is of such volume or character that the mulch functions for only a short time, there will be little effect, either one way or the other, from mulch-forming tillage. Whether evaporation is or is not active naturally has a decided influence on the result. These and other contributing factors must all be taken into account in regulating practice; yet, allowing to each factor its relative importance, the effect of the mulch as a mulch should still be given full consideration.

CONCLUSIONS

(1) The soil mulch has an inhibitory effect on moisture absorption, under conditions where individual rains are not of sufficient volume to fully penetrate the mulch.

(2) The mulch inhibits absorption by increasing the amount of current evaporation in the newly fallen moisture. The volume weight of the stirred mulch being less than that of an equal depth of unstirred soil, the moisture content of the mulched soil immediately after a rain is higher on a percentage basis. When conditions favor evaporation, the result is a greater total loss from the mulched soil. The final moisture content is due to a cumulative effect following several rains.

(3) The soil mulch prevents the loss of moisture already in the soil by checking evaporation.

(4) The practical use of the soil mulch in moisture conservation is

dependent on climatic conditions which influence the prominence of either the inhibitory effect on absorption or the positive effect on retention, or which may nullify either or both.

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FUNDAMENTALS FOR TAXONOMIC STUDIES OF FUSARIUM [1]

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INTRODUCTION

There has been a long-felt need for a general conference of all workers on the Fusarium problem in order that a uniform taxonomy may be formulated upon which future work can be based. The various workers have always been fundamentally in reasonably close agreement regarding the principles of Fusarium classification, but there has been a number of points which were not clearly understood, possibly because the investigators have not been sufficiently in contact with each other. Variations in the methods employed in different laboratories have complicated the problem of species identification. The fact that the fungi vary according to the environment, age, and source of culture and the failure of different workers to understand one another has, it is believed, largely accounted for the differences arising from Fusarium studies.

Recent investigations on the banana-wilt problem conducted by the United Fruit Co. in Central America showed the immediate necessity of a general conference on the Fusarium problem. Because of the large number of related Fusaria encountered in a study of the banana-wilt-producing organism on banana plantations, it was deemed advisable to complete the fungous investigations with a final study in Europe or in the United States. The proposed studies of the United Fruit Co. were discussed at the meeting of scientific societies in Cincinnati, in December, 1923, and through Dr. W. A. Orton a plan for an American conference on Fusarium was proposed. The idea involved the United Fruit Co.'s bringing Dr. H. W. Wollenweber over from Germany, and Dr. O. A. Reinking from Central America. The United States workers included Dr. C. D. Sherbakoff, of the University of Tennessee, Miss Helen Johann and Mrs. Alice A. Bailey, representing, respectively, the Offices of Cereal Investigations, of Cotton, Truck, and Forage Crop Disease Investigations, and of Pathological collections of the United States Department of Agriculture, in a joint study of Fusarium classification. The United Fruit Co., through its director of agricultural research, Dr. John R. Johnston, adopted this liberal policy in supporting the scientific work to a greater extent than their purely economic interests required. It was agreed, on the invitation of Dr. L. R. Jones, to hold the conference at the University of Wisconsin, in Madison.

The purpose of the conference was to give an opportunity to cooperate in a personal way, to compare cultures assembled from all possible sources, and in this manner to clear up the somewhat tangled taxonomy of this difficult genus. All the important European cultures, those being studied at present in the United States, and the collections made from the tropics, primarily from Central America, were assembled at the meeting for special study and comparison. The work of the conference covered, in so far as possible, the study, comparison, and identification of the specimens and cultures of the fungi then available. The main studies were made on the tropical collection, since it was agreed, because of the part taken by the

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United Fruit Co., that the conference should facilitate, in the best way possible, a satisfactory definition of the numerous species found on banana plantations. The studies made of the entire collection embodied species from all sections of the genus Fusarium, including important border-line strains, thereby making it possible to arrive at a uniform taxonomy of the group. It is believed that the main points of difference have been agreed upon and that the present paper will present a clearer understanding of the Fusarium problem from the standpoint of the identification of species.

Material of each species studied and identified at the conference will be deposited in the pathological collections herbarium of the Bureau of Plant Industry, United States Department of Agriculture, for permanent preservation. The cultures will be prepared in the manner most satisfactory for the preservation of herbarium material. Pure cultures of each of the species studied and identified will also be placed in the bureau. These cultures will have substantially the status of type specimens.

CRITERIA OF THE NORM

Only a résumé of the criteria of the norm will be here considered, since a complete discussion of the subject has appeared in former publications (*5*, *9*).[5] The normal condition of Fusaria from the standpoint of determination may be present in nature, but generally it must be produced by growing the fungi under pure culture conditions. A few Fusaria such as *F. dimerum* Penz., *F. scirpi* Lamb. et Fautr., *F. culmorum* (W. G. Sm.) Sacc., and some others may be determined directly from the fungus growing under natural conditions without resorting to pure cultures. Other Fusaria, which appear primarily in a microconidial stage under natural conditions and produce only a few sickle-shaped spores, are more difficult to identify. For these the so-called normal growth must, at first, be produced. It is, therefore, necessary to make a careful study from pure culture in order accurately to identify these organisms. Generally, macroconidia are regarded as the normal spore type. In certain groups, however, the microconidia may have definite characters, such as a pearlike shape or a formation in chains, which aid in the determination of the section (Sporotrichiella, Arthrosporiella) and, in exceptional cases, may even lead to the identification of species (*F. poae* (Peck) Wr., *F. moniliforme* Sheld., *F. decemcellulare* Brick.).

Other normal reproductive stages, such as chlamydospores, by their presence or absence indicate the border of certain groups (Elegans, Lateritium). Sclerotia may be characteristic for groups (Lateritium) and even for a number of species (*F. sclerotium* Wr.). Color of the conidia and color of the aerial mycelium and stroma are further reliable characters for taxonomy. Minor characters such as mycelium, hyphae with specialized cells, coremia-like aggregations of hyphae, and aromatic odors may also be of some importance for differentiation. In opposition to the norm there are abnormal features which might be valuable to mention in the diagnosis. Not so much stress, however, is placed on degenerated conditions in the life cycle of Fusaria.

[5] Reference is made by number (italic) to "Literature cited," p. 843.

FIG. 1.—Stages in the life cycle of Fusaria:
A–F, H and S, microconidia.
I–R, T–X, macroconidia.
Y, Swellings of hyphae.
Z, A_1–D_1, Chlamydospores.
E_1–F_1, Conidial propagation from conidia.
G_1, Sclerotium.
H_1, Sporodochium.
I_1, Sclerotia in clusters on an erumpent base.
K_1, Columns formed by conidia borne in chains.
L_1–N_1, Pionnotes covering either the medium directly (L_1), or a mycelial sheet (M_1), or numerous associated sporodochia (N_1) formed on the substratum.

Examples: *Fusarium poae* (Peck) Wr. (A, J), *F. orthoceras* App. et Wr. (B, E, K, A_1, B_1), *F. moniliforme* Sheld. (C, F, G, G_1), *F. chenopodinum* (Thüm.) Sacc. (D, M, N, Z), *F. argillaceum* (Fr.) Sacc. (H, I, C_1, D_1), *F. subcarneum* Crouan (L), *F. flavum* (Fr.) Wr. (O), *F. dimerum* Penz. (P), *F. salicis* Fuck. (Q), *F. sarcochroum* (Desm.) Sacc. (R, S), *F. viticola* Thüm. (T), *F. solani* (Mart. pr. p.) App. et Wr. (U), *F. vasinfectum* Atk. (V, normal, W, swollen, X, dried spore), *F. tricinctum* (Cda.) Sacc. with swellings of hyphae (y), *F. macroxysporum* Lindf. with pionnotal propagation of macroconidia (E_1) and development of microconidia (F_1).

For a proper understanding of the terms (*8*) used for normal forms of reproductive stages and mycelium of Fusaria the characters considered of primary importance are enumerated below.

I. Normal reproductive stages

1. *Microconidia:* 0–(0–3)–septate, globose-pearshaped as in *F. poae* (Peck) Wr. (fig. 1A); ellipsoidal as in *F. orthoceras* App. et Wr. (fig. 1, B); ovoid-fusoid as in *F. moniliforme* Sheld. (fig. 1, C); comma-shaped as in *F. chenopodinum* (Thüm.) Sacc. (fig. 1, D). They may be found scattered (fig. 1, A–D), in false heads (fig. 1, E), or in chains (fig. 1, F) and may form sporodochia and pionnotes.

2. *Macroconidia:* 0-pluriseptate, dorsiventral, fusoid to sickle-shaped, apedicellate and pedicellate, scattered, sporodochial and pionnotal.

   a. Septation types:
      0–septate, *F. flavum* (Fr.) Wr. (fig. 1,O);
      1–septate, *F. dimerum* Penz. (fig. 1, P);
      3–septate, apedicellate, *F. betae* (Desm.) Sacc. (fig. 1, K);
      3–septate, pedicellate, *F. poae* (Peck) Wr. and (fig. 1, J);
      5–septate, *F. chenopodinum* (Thüm.) Sacc. and (fig. 1, M);
      5–7–septate, *F. chenopodinum* (Thüm.) Sacc. and (fig. 1, N);
      9–septate, *F. decemcellulare* Brick.

   b. Sporulation types: Scattered; in false heads; pionnotes, continuous, gelatinous, slimy, (*1*) formed on the naked surface of the substratum (fig. 1, L_1) as in section Eupionnotes, species of section Roseum, Gibbosum, Elegans and Discolor, or (*2*) on a mycelial sheet as in *F. avenaceum* (Fr.) Sacc. (fig. 1, M_1) or (*3*) composed of an aggregation of sporodochia as in *F. martii* App. et Wr. (fig. 1, N_1); sporodochia, (*1*) tubercularialike (fig. 1, H_1) found in the majority of species of all sections except Ventricosum and some species of Elegans and Eupionnotes (*2*) in columns representing a chainlike development, as in *F. stilboides* Wr. (fig. 1, K_1).

   c. Shape: Fusoid-lanceolate, slightly curved, mostly apedicellate, *F. argillaceum* (Fr.) Sacc. (fig. 1, I); slightly sickle-shaped, apedicellate, *F. betae* (Desm.) Sacc. (fig. 1, K); sickle-shaped, pedicellate, most of the species; strongly dorsiventral, *F. scirpi* Lamb. et Fautr. and *F. chenopodinum* (Thüm.) Sacc. (fig. 1, N); terminal cell suddenly constricted, *F. culmorum* (W. G. Sm.) Sacc. and *F. chenopodinum* (Thüm.) Sacc. (fig. 1, M); terminal cell whiplike, much elongated *F. scirpi* Lamb. et Fautr. and *F. chenopodinum* (Thüm.) Sacc. (fig. 1, N); curvature within the limits of conical sections as elliptic (fig. 1, M), parabolic and hyperbolic (fig. 1, N). The curvature is mainly determined from the dorsal and ventral sides of spores, in side view, within the septation regior.

3. *Chlamydospores:* Terminal (fig. 1, B_1, D_1), intercalary (fig. 1, A_1), single, in chains, in clusters, mycelial (fig. 1, Z to B_1), conidial (fig. 1, C_1).

   a. Terminal: In sections Elegans, Martiella, Ventricosum; unicellular, *F. argillaceum* (Fr.) Sacc. (fig. 1,N D_1); 0–1-septate, sections Martiella and Elegans (fig. 1, B_1). Most fungi forming terminal spores may also develop intercalary spores. In section Ventricosum no intercalary spores are produced.

   b. Intercalary: Mostly 0–1-septate (fig. 1, A_1) in sections Elegans, Martiella, Discolor, Gibbosum, and Eupionnotes (subsection Chlamydospora). Chains and clusters (fig. 1, Z) may also occur in most of these sections.

4. *Sclerotia:* Plectenchymatic structures, general appearance similar to perithecia of Gibberella, but with uniform structure throughout. Globose or rugose, single or in cauliflowerlike clusters (fig. 1, G_1). Color, blue in sections Roseum, Gibbosum, Lateritium, Discolor, Elegans, and Martiella; brownish to dark brown in Arthrosporiella (*F. diversisporum* Sherb.), Gibbosum, and some species of section Discolor.

II. Color characters

1. *Color of conidia:* Brownish white to golden yellow in section Martiella; brownish white to pale orange in section Elegans, Discolor, Gibbosum, and Arthrosporiella; orange in section Roseum Lateritium, Eupionnotes and Arachonites. The color of conidia is sufficiently constant to be a reliable character for some groups.
2. *Color of aerial mycelium:* White, rose to yellow and blue, mostly representing a diffused color of the stroma. Changes in color of mycelium due to the reaction of the substratum is substantially the same as that discussed under color of stroma.
3. *Color of stroma:* Brownish white, carmine, yellow, and blue. The acid modification is especially well developed on sterilized rice and may aid in the identification of Fusarium groups. This acid modification differs in some sections for it is golden yellow in most of the species in section Discolor, Saubinetii, Roseum, Sporotrichiella, and in some of Gibbosum; rose to vine red in many species of section Elegans; and rose to brown color, with a tendency toward diffusing into rice, in section Martiella. The basic color modification is mostly blue or violet. In general, all of these fungi produce on rice an acid color modification that may change gradually with age into an alkaline modification. This color change can be produced at once by the addition of sufficient alkali to a well-developed culture of the fungus on rice. Other characteristic colors produced on sterilized potato tubers are carmine red by species of section Discolor, Roseum, Sporotrichiella, and some of Gibbosum, and citric or sulphuric yellow in Neesiola subsection of Discolor. These colors are of the basic modification and consequently are not turned blue by the addition of alkali. The carmine red turns yellow with an addition of acid. No color contrast is observed in sections such as Eupionnotes, Arachnites, and Ventricosum. The stroma and aerial mycelium of these fungi have the color shades of their conidia. The color of sclerotia and sclerotial stroma is blue in some and brown in other groups, as discussed under sclerotia.

III. Minor characters

1. *Hyphae with swollen cells:* Not true chlamydospores (fig. 1 Y) as in *F. flocciferum* Cda. and *F. tricinctum* (Cda.) Sacc.
2. *Aerial mycelium:* Loose, dense, jellylike, cottony, radiate, zonate.
3. *Immersed mycelium*: Slimy or leathery sheet (stroma), that may or may not have a plectenchymic base. Section Eupionnotes.
4. *Coremialike aggregations of hyphae.* Aggregations of anastomosed hyphae as in Eupionnotes and Ventricosum. True coremia so far were not observed.
5. *Aromatic odor:* Somewhat similar to lilac odor, produced by a number of species of section Elegans such as *F. oxysporum* Schlecht., *F. hyperoxysporum* Wr., *F. zonatum* (Sherb. s. var.) Wr., *F. vasinfectum* Atk., and *F. cubense* Erw. F. Sm. The last three species have the strongest odor, especially when grown on rice.

IV. Abnormal characters

1. *Spore:* Exceptional size, irregularities in shape of conidia, swollen cells in germinating spores (fig. 1, W) and constricted cells in dry conidia (fig. 1, X). These abnormal characters, although frequently present, are not taken into consideration for taxonomic purposes and have been more fully discussed elsewhere (*1*).

2. *Mycelium:* With the exception of Eupionnotes, in which immersed growth of the mycelium predominates even under normal and favorable spore production, changes to an immersed gelatinous growth in general indicate degeneration and self-digestion accompanied by abnormal spore production or even sterility. This condition may be changed into the norm by transferring to various media or by selection of virulent aerial colonies from new agar plates. Fortunately the number of Fusaria showing a tendency to degenerate seems to be small. This tendency is notable in *Fusarium nivale* (Fr.) Sacc., *F. anthophilum* (A. Br.) Wr., *F. orthoceras* App. et Wr., and *F. flocciferum* Cda. The latter two fungi if inoculated into living potato tubers, thoroughly disinfected with formaldehyde, regain their normal behavior when reisolated and retransferred to sterilized media. In some cases, agar media too rich in sugar or too alkaline or acid in reaction favor degeneration. Conditions, however, that favor normal conidial production generally warrant longevity and sufficient constancy. On the same medium mycelium transfers often give only a sterile stroma, while a transfer of macroconidia increases the tendency to reproduce this stage. These facts if not fully understood may make the proper identification of certain species doubtful. They show the necessity of improving cultural methods.

Brown and Horne (*3*) in their studies of the genus Fusarium have given interesting details on the modifying effect of transfers from various parts of a given fungus. These authors have stated that the rate of spread of certain colonies fell off after a time and was reduced (staling), while other colonies developed into normal sporodochia or pionnotes with small and low septate or long and high septate conidia. Furthermore, "saltants" showing sectorial effects of some strains have been produced in Fusaria derived from six different isolations from apple. In the preliminary studies this wide range of variability was considered to be connected with one and the same fungus, *F. blackmani* Brown et Horne.

This variability of the mycelium and spore character has been found to occur with various fungi. Notable among these are two closely related Fusaria described as *F. anguioides* Sherb. and *F. anguioides* var. caudatum Sherb. (*5*). These two organisms are closely related to *F. anthophilum* (A. Br.) Wr. isolated from apple fruit in England. The abnormal variations found in these cases, though great, were not mentioned in the diagnosis of the Fusaria because normal characters were regarded as sufficient for determination.

PRODUCTION OF THE NORM

Cultural conditions which will produce good mycelial growth are not necessarily those most favorable for the production of normal spores. In the study of Fusarium it has been found necessary to use a variety of media for the production of the various normal mycelial and reproductive stages. The best media for these purposes at present available are the ordinary ones used in plant pathology investigations. A combination of a number of vegetable substances may prove valuable in some cases, but, so far, sufficient work has not been done to justify recommending such a medium. Because the same vegetable under different conditions varies in its chemical composition, it would be highly desirable to produce a satisfactory synthetic medium. It is hoped that a synthetic nutrient may sometime be found which will render it possible to control the production of spore bodies or sclerotia at will. Until some such new media are produced, vegetable media will be found to be fairly satisfactory for taxonomic purposes.

No one culture medium is at present used which will produce optimum development of all phases of fungus growth; hence certain media have been selected which proved most satisfactory for the best production of conidia, and other media for the production of sclerotia, mycelium, chlamydospores, and other characters. It must be understood, however, that not all species will react in exactly the same way, and therefore a medium which produces normal conidia in most species may need to be supplanted by another in the case of other species. The media used at the conference are given in Table I.

TABLE I.—*Culture media showing types of growth readily produced on each.*[a]

Media	Conidia	Chlamydospores	Sclerotia	Mycelium	Color
Potato tuber cylinders (no water added)	+	+	+	+	+(basic)
Oatmeal agar (Sherbakoff (5))	+		+		+(basic)
Potato agar+2 per cent dextrose (200 gm. to 1,000 c. c.)	+	+	+		
Potato agar+5 per cent dextrose (200 gm. to 1,000 c. c.)	+		+	+	+(basic)
Rice (2 gm. to 6 c. c. water)	+		+		+(acid)
Melilotus stems (mature stems+4 c. c. water)	+	+	+		Color of spores
Lupinus stems (mature stems+4 c. c. water)	+		+		Color of spores
Alnus (2 to 3 year twigs+4 c. c. water)	+		+		Color of spores

[a] All agar media contains 2 per cent agar. All media are autoclaved for 45 minutes at 10 pounds pressure except rice, which is steamed for one hour on three successive days. When necessary to use young Melilotus stems, 4 c. c. of a 0.5 per cent KOH solution should be used in place of water to neutralize the acidity of the stems.

In general, the use of several vegetable media, such as those mentioned in the table, will result in the production in good condition of the various characteristics of the fungus. In some cases special methods must be employed for the production of certain phases. The addition of acid to the standard potato agar greatly stimulates an abundant porduction of normal spores in such species as the Fusarium stage of *Gibberella saubinetii* (Mont.) Sacc. Substances poor in food value favor the production of chlamydospores. In species which produce chlamydospores only rarely, as *F. aurantiacium* (Lk.) Sacc., chlamydospore development may be brought about by growing in sterile tap water. The reaction of the medium is important from the standpoint of color production and, in some cases, greatly influences growth of mycelium and abundance of spore production.

In transferring, it must be borne in mind that the type of inoculum influences the resultant growth. Continuous transfer of one type of inoculum tends to production of that type of growth. Repeated transfer of mycelium or chlamydospores tends to good development of mycelium and sclerotia in those species which have sclerotia. For production of abundant spores, transfers should be made from sporodochia or pionnotes. When sporodochia are lacking and macrospores are not numerous, the chance of transferring mycelium may be eliminated by making dilutions in tubes of sterile water and transferring a loop full of the dilution to the culture medium, or by using the plate dilution method. The latter method affords an opportunity to select colonies showing the greatest tendency toward spore production.

Environmental factors other than the medium which should be taken into consideration are temperature, humidity, and light. There are indications, in some species at least, that temperature has an appreciable effect upon the morphology of the conidia as well as upon the rate of growth of mycelium (*4*). Perhaps to temperature may also be ascribed part of the effect on color intensity usually attributed to light. Humidity affects the nature of the culture, as is evidenced by the increased production of chlamydospores in some species and the production of swollen conidia in excessively moist media. The fact that these environmental factors have been given very little consideration in identification of Fusaria may be responsible for some of the difficulties encountered in various countries where taxonomic work is done.

IDENTIFICATION

If we realize the fact that tubercular sporodochia with normal and uniform conidia occur in the majority of Fusaria that can be easily grown in pure culture, there will be no difficulty in judging the normal stages and, consequently, in identifying most of these fungi. For the remaining forms more detailed studies will have to be conducted in order to produce or prove the absence of particular stages such as sporodochia, pionnotes, chlamydospores, and sclerotia. It should be stated also that even conidia normal in appearance often differ greatly in their size and shape according to where they are produced, whether on mycelium over a wet surface or in a definite sporodochium. In a typical pionnotes long conidia may be produced, and in sporodochia they may be considerably shorter, as in *F. vasinfectum* Atk., *F. cubense* Erw. F. Sm., *F. lycopersici* (Sacc.) Wr., and others. Differences in size and shape may also appear when the fungus is grown on

various types of media. In section Roseum longer and slenderer conidia are produced on rice and oatmeal agar than on other media more favorable to sporodochial development. Because of these differences, it is necessary to pay particular attention to the average size of different types as grown upon different media and under different conditions. A comparison must be made only of conidia produced under comparable conditions and between comparable sporulation types.

Often the identification is difficult because certain species persistently produce only a microconidial stage. In such instances some special methods must be employed to induce the fungus to develop macroconidia. For instance, in the case of *F. chenopodinum* (Thum.) Sacc. only the comma-shaped spore (fig. I, D) may be observed for a long time. However, by repeated transfers of occasionally found macroconidia, finally normal macroconidia can be obtained in abundance. These conidia enable us to identify the fungus with ease. It is important to emphasize that, in this species as in some others, often an intermediate type of macroconidia is produced when the fungus is in a semimycelial stage of growth and when sporodochia are underdeveloped. These conidia in *F. chenopodinum* (Thum.) Sacc. can easily be taken for those of *F. sambucinum* Fuck. Generally the microconidia in themselves do not represent definite enough characters to be used for identification.

In those doubtful cases where normal macroconidia are not readily produced the presence of chlamydospores may be of extreme importance for determining the group. The presence or absence of chlamydospores makes it possible to separate species of section Elegans with terminal chlamydospores from section Lateritium with no chlamydospores, even though their macroconidia may be similar when grown under certain cultural conditions.

The presence of ascigerous stages in some of the sections is of additional help in the identification of the imperfect or conidial forms (*9*, *11*). A number of ascomycetes (Nectria, Calonectria, Hypomyces, Gibberella) have been grown from the ascospores in pure culture and developed the conidial stage, thus showing the relationship. In other cases cultures of Fusaria collected from nature have developed perithecia.

Color characters on various media rich in carbohydrates afford a fairly reliable means of placing the fungi into sections. The color of conidia and stroma especially represent an important complex for identification.

The ordinary procedure in determining Fusaria is to illustrate and measure normal spores and a few exceptions that show the variability and changes with increasing age. The standard magnification used for the drawings is 1–1000 for the spores and 1–500 for parts of sporodochia showing conidiophores and formation of spores. Smaller magnifications may be used for special purposes, such as groups of sporodochia, sclerotia, and stroma erumpent. For a short study water mounts are preferable. In order to prevent moisture from escaping during the procedure of drawing, the edges of the cover glass may be waxed. Another method (*7*) of preparing slides, which has the advantage of placing all the spores in one plane and holding them in place, is to make the mount on a very thin (0.5 mm.) sheet of agar placed on the slide. These agar sheets can readily be prepared by pouring clear agar between glass slides set the required distance apart. This method is very convenient for photographic purposes.

CLASSIFICATION OF THE FORM-GENUS FUSARIUM

The form-genus Fusarium includes all hyphomycetes and conidial stages of ascomycetes that have no black or pure gray color either in mycelium or in conidia and that have macroconidia that are acrogenous, typically septate, sickle-shaped, and not round at the ends. Microconidia, chlamydospores, and sclerotia may be present. Some Fusaria have been proved to be conidial stages of certain Hypocreaceae, such as Nectria, Hypomyces, Gibberella, and Calonectria. The fungi of the form-genus Cylindrocarpon, formerly considered as Fusaria, have Nectrias and certain Hypomyces (*8*) as perfect stages. Some of the species of Ramulara or similar fungi as Septomyxa also formerly placed under Fusarium, have as their perfect stage Neonectria (*10*, *11*) and Mycosphaerella (*11*). The fungi parasitic on scale insects and possessing sickle-shaped to fusoid conidia are referred to the genus Microcera, which is said to be the conidial stage of Sphaerostilbe.

GROUPING OF FUSARIA IN SECTIONS

Many Fusaria readily fall into separate groups possessing similar characters. These groups are called sections, which, on the basis of their apparent

relationship, especially when their perfect stages are considered, are arranged as follows: 1, Eupionnotes Wr.; 2, Arachnites Wr.; 3, Sporotrichiella Wr.; 4, Camptospora Wr.; 5, Arthrosporiella Sherb.; 6, Gibbosum Wr.; 7, Roseum Wr.; 8, Liseola n. n.[6]; 9, Lateritium Wr.; 10, Discolor Wr.; 11, Spicarioides N.comb.[7]; 12, Saubinctii Wr.; 13, Elegans Wr.; 14, Martiella Wr. (sensu extenos)[8]; and 15, Ventricosum Wr.

KEY TO THE SECTIONS OF FUSARIUM

With the exception of section Camptospora (*10*), which needs further study, all of the species are arranged in the following key to the sections:

a. Microconidia on aerial mycelium usually present and dominately 0-septate, ovoid, fusoid, reniform, or pearshaped.
  b. 0-septate conida pearshaped. Macroconidia when normal are in shape intermediate between those of the sections Elegans and Roseum, though more curved than either; substratum rose-colored, intercalary chlamydospores, or similar structures may be present. Sec. 3, *Sporotrichiella.*
  bb. 0-septate not pearshaped.
    c. 0-septate conidia in chains.
      d. Conidial walls thin. Microconidia mostly in chains, fusoid-ovoid; macroconidia in form and color similar to those of sec. Lateritium; no chlamydospores; substratum vinaceous-violet; some of the species are connected with Gibberellas of sec. Lisea (Sacc.) Wr.....Sec. 8, *Liseola* n. n.
      dd. Conidial walls thick or highly refractable. Macroconidia pluriseptate, in shape resembling those of the sec. Discolor.....Sec. 11, *Spicarioides* n. comb.
    cc. 0-septate conidia not in chains.
      d. Conidial walls thin. Macroconidia attenuate at the top ends, pedicellate; terminal and intercalary chlamydospores present; color of conidia brownish to salmon; no blue or green color in conidia even as a diffusion from stroma; stroma on artificial media principally vinaceous to lilac.....Sec. 13, *Elegans.*
      dd. Conidial walls relatively thick. Macroconidia somewhat truncate or rounded at the top end, or at least not distinctly attenuate, some times slightly constricted at the tip ends; terminal and intercalary chlamydospores present; color of conidia brown-white to golden brown with occurrence of green to green-blue as diffusion from stroma.....Sec. 14, *Martiella.*
aa. Microconidia on aerial mycelium usually absent or 0-3 or more septate, reniform, comma, spindle, to sickleshaped.
  b. Macroconidia apedicellate. Color type, orange to light salmon.
    c. Typical pionnotes always present; comparatively slow-growing fungi.....Sec. 1, *Eupionnotes.*
    cc. Typical pionnotes absent; comparatively fast-growing fungi.....Sec. 2, *Arachnites.*
  bb. Macroconidia subpedicellate to pedicellate.
    c. Terminal chlamydospores present; intercalary chlamydospores absent; no true sporodochia; macroconidia wedge-shaped to slightly sickle-shaped, not constricted at the top. Sec. 15, *Ventricosum.*

[6] Liseola nn. (Syn. Constrictum Wr. pro parte subs. Elegantis; Moniliforme Sherb.*b*); Microconidiis plus minusve in catenulis, dispositis, fusoideis-ovoideis, macroconidiis forma et colore sectionis Lateritii, liberis, in sporodochiis, in pionnote; chlamydosporis nullis; stromaet violaceo. Status conidicus Gibberellarum sect. Liseae (Sacc.)

[7] Wr. Spicariodes (Wr. subsect.) n. comb. (Syn. subsect Spicarioides sectionis Discoloris Wr.) Microconidiis sporodochialibus pluriseptatis forma specierum sectionis Discoloris. Chlamydosporis nullis Stromate carmineo.

[8] *Martiella* Wr. (Sensu extenso sectionis Martiellam Wr. et Pseudomartiellam Wr. includendo). Macroconidiis dorsiventralibus fusoideofalcatis, apice rostrato truncato vel rotundato, basi plus minusve subpedicellata, in sporodochiis et pionnote sordide albo, ochroleuco vel aureo; stromate aerugineo fere nigrescenti, chlamydosporis terminalibus, intercalaribus, singulis, binis, catenulatis vel acervalibus. Status conidicus Hypomycetum sect. Pseudomartiellae.

cc. Terminal chlamydospores absent.
d. Intercalary chlamydospores present.
e. Sporodochia typically absent. Conidia, when free-borne on aerial mycelium, spindle-shaped. Macroconidia gradually attenuate, generally lanceolate, apedicellate; but also sickleshaped and pedicellate; color intermediate between that of Roseum and Gibbosum sections, sclerotia may be present____Sec. 5, *Arthrosporiella.*
ee. Sporodochia typically present.
f. Macroconidia with top ends much attenuated; stroma typically brown,[9] sometimes carmine[10]_____Sec. 6, *Gibbosum.*
ff. Macroconidia with top ends somewhat truncate; conidia ochreous to salmon; stroma Roseum-like; blue sclerotia may be present_____Sec. 10, *Discolor.*
dd. Intercalary chlamydospores absent.
e. Top ends of macroconidia gradually attenuate; when free-borne on aerial mycelium, sickle-shaped; or none. Acid-color modification of aerial mycelium yellow except in *F. anthophilum* (A. Br.) Wr., and other related fungi; conidial walls thin_____Sec. 7, *Roseum.*
ee. Top ends of macroconidia somewhat constricted.
f. Conidial walls thin, and in this character, as well as in shape and color, similar to section Elegans_____Sec. 9, *Lateritium.*
ff. Conidial walls thick, highly refracable, and in this character, as well as in shape and color, similar to section Discolor; conidial stage of *Gibberella saubinetii* and other similar Gibberellas____Sec. 12, *Saubinetii.*

RELATIONSHIP OF FUSARIA TO ASCOMYCETES

A number of different species of Fusaria have been definitely connected with certain ascomycetes of the Hypocreales group. In some other cases the connection is very probable, and in still other cases the connection is suggested as possible. For convenience the instances are mentioned here under different sections of the genus Fusarium.

Sec. 1. Eupionnotes. Perfect stage, *Nectria moschata* Glück, conidial stage similar to *F. aquaeductuum* Lagh. var. *pusillum* Wr.

Sec. 2. Arachnites. Perfect stage, *Galonectria graminicola* (Berk. & Brome) Wr., conidial stage *F. nivale* (Fries) Ces.

Sec. 3. Sporotrichiella. No connection with an ascomycete is known.

Sec. 4. Camptospora. Perfect stage *Nectria episphaeria* (Tode) Fr., conidial stage may be *F. cavispermum* Cda.

Sec. 5. Arthrosporiella. No definite connection with an ascomycete is known.

Sec. 6. Gibbosum. No connection with an ascomycete is known.

Sec. 7. Roseum. *Gibberella tropicalis* Rehm. possibly has Roseum-like conidial stage.

Sec. 8. Liseola. *Gibberella acervalis* (Moug.) Wr., conidial stage very similar to *F. moniliforme* Sheld.

Sec. 9. Lateritium. *Gibberella baccata* (Wallr.) Sacc., conidial stage *F. lateritium* Nees; *G. pulicaris* (Fr.) Sacc., conidial stage *F. sarcochroum* (Desm.) Sacc.; *G. moricola* (Ces. et Not.) Sacc., conidial stage *F. urticearum* (Cda.) Sacc.; *G. effusa* Rehm, conidial stage *F. salicis* Fuck.; *G. evonymi* (Fuck.) Sacc., conidial stage *F. pyrochroum* (Desm.) Sacc.; *G. juniperi* (Desm.) Wr., conidial stage *F. fructigenum* Fr.

Sec. 10. Discolor. *Gibberella heterochroma* Wr., conidial stage *F. polymorphum*-like; and *G. cyanogena* (Desm.) Sacc., conidial stage *F. sambucinum* Fuck.

Sec. 11. Spicarioldes. No connection with an ascomycete is known.

Sec. 12. Saubinetii. *Gibberella saubinetii* (Mont.) Sacc., conidial stage *F. graminearum* Schwabe; *G. flacca* (Wallr.) Sacc., conidial stage *F. caricis* Oud.

[9] Subsection Eugibbosum n. subsect.
[10] Subsect. Ferruginosum (Sect. Ferruginosum Sherb.) n. comb.

Sec. 13. Elegans. No connection with an ascomycete is known.
Sec. 14. Martiella. *Hypomyces ipomoeae* (Hals.) Wr., conidial stage *F. javanicum* Koord.; *Hypomyces cancri* Wr., conidial stage *F. striatum*-like; *Hypomyces leptosphaeriae* (Niessl) Wr., conidial stage *F. sphaeriae* Fuck.
Sec. 15. Ventricosum. *Hypomyces solani* Rke. et Berth., conidial stage *F. argillaceum* (Fr.) Sacc.

NOMENCLATURE IN RELATION TO SPECIES, VARIETIES, AND FORMS

The plan recently considered and approved by the committees of the Phytopathological Society, the Society of Agronomy, and the Mycological section of the Botanical Society on June 6, 1924, in Washington, D. C., is followed in regard to questions of nomenclature and terminology. This plan provides for the use of the Latin trinomial composed of the genus, species, and variety. The *species* includes groups of individuals which can be separated on the basis of morphological character of such a nature as to be applicable and usable by mycologists in general and which will be most serviceable for practical purposes. The *variety* is distinguished by morphological characters, but less important than those used for specific segregation. An additional category termed *forma* is to be applied to subdivisions of the species or varieties characterized and distinguished primarily by physiological instead of morphological characters, though in some instances there may be present also some slight morphological, distinguishable differences. The *forma* is designated by an arabic numeral.

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THE HAM BEETLE, NECROBIA RUFIPES DE GEER[1]

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INTRODUCTION

The principal species of insects and mites which damage smoked meats fall into two rather distinct groups: (1) Those that infest newly smoked, juicy meats—the cheese skipper (*Piophila casei* L.) and several species of blowflies (*Lucilia sericata* Meig., etc.,); (2) those that infest meats which have become dried to some extent by evaporation during long storage or as a result of prolonged smoking, or both—the ham beetle (*Necrobia rufipes* De G.), the larder beetle (*Dermestes lardarius* L.), the leather beetle (*Dermestes vulpinus* Fab.), and certain mites. The species described in this paper is the most important of the second group, and it is sporadically very injurious where smoked meats are stored for rather long periods. A large part of the expense of protecting cured meats with wrappings, sacks, and washes may properly be charged to this insect.

Riley (*24*)[2], who made the first economic investigation of the insect 50 years ago, cited cases of extensive injury to hams in St. Louis and Boston. In the dispute arising because of the infested stocks at Boston the consignee claimed that the husk paper in which the consignor had wrapped the meat was likely to generate the worm. The referees of the case deposed, however, that "a warm, damp atmosphere and want of free circulation of air on the hams will produce or generate the worm in light-salted sugar-cured hams."

During the summer of 1921 a severe infestation developed in dry-cured Army bacon stored in crates at Baltimore and later at Washington. There were about 220,000 pounds of this bacon, and it was reconditioned by extensive trimming, which in many cases reduced the weight of sides by 75 per cent.

Howard (*16, p. 105–107*) defined the status of the pest as it is at present when he stated, in 1902, "that it is hardly a species which causes a constant drain on the trade, but occasionally becomes extremely abundant, ruining large quantities of cured meats."

SYSTEMATIC POSITION, SYNONYMY

This species is the most injurious of the coleopterous family Cleridae, the larvae of which are typically predacious and often beneficial as enemies of economic insects, including the tobacco beetle (*Lasioderma serricorne* Fab.) and many species which attack forest trees.

De Geer (*12, p. 165*) published the original description as *Clerus rufipes* in 1775. In 1796 Latreille (*18, p. 35*) erected the genus Necrobia. Mulsant and Rey (*22, p. 122–124*), who placed the species in the genus Agonolia, listed *Clerus rufipes* De Geer, Oliv., *Dermestes rufipes* Fab., *Corynetes rufipes* Herbst., etc., *Necrobia rufipes* Oliv., etc., as indicating the four genera to which the present species had been referred:

The following specific references (*27, p. 142–143*) are synonymous with *rufipes:*

amethystina Steph., 1832, Ill. Brit. Ent. 5: 417; Klug, 1842, Clerii, Phys. Abh. K. Akad. Wiss. Berlin for 1840, p. 394.

dermestoides Pill. et Mitterp., 1783, It. Poseg., p. 68, pl. 7, fig. 8.

foveicollis Schklg., 1900, Mitt. Nat. Mus. Hamburg 17: 20.

glabra Champollion, 1814, Millin Mag. Encycl. 3: 44; 1902, Schenkling, Bul. Mus. d'Hist. Nat. 8:332.

mumiarum Hope, 1834, Pettigrew, Hist. Egypt. Mum., p. 54, pl. 5, figs. 1–3; Schenkling, op. cit., p. 332.

pilifera Reitt., 1894, Verh. Nat. Ver. Brünn 32: 85; Abeille, 1895, Bul. Soc. Ent. France 1: 208.

COMMON NAMES

The common name "red-legged ham beetle" was given to the insect by Riley (*24*) in 1874. An article in the Yearbook of the United States Department of Agriculture for 1907 (*1, p. 552*) referred to the pest as the "ham beetle." Dealers in meats know the insect by the name "paper worm."

[1] Received for publication April 22, 1924; issued June, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 863.

According to Froggatt (*10, p. 26*) *N. rufipes* is known in the Pacific Islands as the "copra bug."

Inasmuch as the other beetles which sometimes attack smoked pork already have well-established common names—"larder beetle" and "leather beetle"—the writers prefer the name "ham beetle" for *Necrobia rufipes* De Geer.

THE ADULT

TECHNICAL DESCRIPTION

Form oval, sides subparallel, widest at apical fourth. Color blue, sometimes with violaceous or greenish luster, legs and first five segments of antennae castaneous, terminal six segments of antennae and the trophi piceous, eyes black, venter aeneous black. Head rather sparsely punctured, the punctures large and small intermingled, median portion of frons and vertex with very few punctures. Pubescence sparse, erect, and black. Eyes finely granulated, with a triangular emargination approximate to the antennal insertion. Labrum emarginate. Both maxillary and labial palpi with subcylindrical, slightly acuminate terminal segments. Antennae each with eleven segments; the first thick, slightly bent; the second about one-third the length of first, equilateral; third almost twice the length of second but of equal thickness; fourth to eighth mutually equal in length, each just perceptibly wider than the one preceding; the eighth is transverse; ninth and tenth strongly transverse, subequal, about one-half as long as broad; the eleventh almost square. Pronotum transverse, sides evenly curved from base to apex, basal and apical angles very obtuse, almost wanting, lateral cariniform margin distinct, finely serrulate. Surface rather sparsely set with moderately coarse punctures; punctures much more dense at sides than on disc; pubescence as on head. Scutellum small, transverse. Elytra long, suture closed, lateral margin finely beaded, each with nine distinct rows of punctures, the normally occurring tenth row being confused with the ninth; rows obsolete just behind the middle, surface between puncture rows and of apical portion rather densely set with fine punctures from each of which a posteriorly-directed subrecumbent black hair arises. Under parts and legs rather finely and densely punctured, clothed with pale fulvous pubescence with a few longer black hairs interspersed. Legs moderately long, femora not greatly enlarged, tibiae straight, tarsi short, of five segments of which the fourth is very small and concealed between the lobes of the third, first three segments with lamelliform pads beneath. Claws rather long, provided at base with a broad toothlike appendage. Length: 3.5 to 7 mm.[3]

In the female each of the elytral punctures, which are arranged in rows, gives rise to a stiff black hair slightly inclined anteriorly; in the male these hairs are subrecumbent and directed posteriorly. Other secondary sexual characters are absent.

ADULT BEHAVIOR

After the transforming insect becomes adult, it gnaws an irregular hole in the wall of the pupal cell, and emergence occurs. The meconium is voided in the cocoon. Sometimes a day or two elapse between the time the adult becomes fully pigmented and its escape, and in the case of adults emerging in vials they often return to the cell for concealment.

Mating usually occurs promptly after two newly emerged beetles of opposite sex are placed together, and is frequently observed during the long oviposition period, especially when the beetles appear frightened during manipulation of the dishes in which they are confined. The forwardly directed elytral spines of the females doubtless materially assist the males, which are usually smaller than the females, in maintaining their position during copulation.

Besides sharing in the attacks of the larvae upon ham and cheese, the adults are markedly predacious and also cannibalistic. It is apparent that the beetles and their larvae can destroy an infestation of cheese skippers (*Piophila casei* L.) in ham,[4] and under some conditions, as in stores of bones, they probably are beneficial to the extent that they help destroy the maggots of skippers and blowflies. In the laboratory experiments fat bacon was a much less favored food for adults than skipper larvae.

Even when fed daily with skipper maggots, ham beetles sometimes dismember an individual of their own species and then proceed to devour it. Specimens which die naturally are usually promptly eaten. The beetles have also been observed by the writers to eat the eggs and larvae of their own species, and where adults become very numerous, as has occurred in boxes of smoked meat under observation, their cannibalism is apparently responsible for a great reduction in the number of their larvae. Adults which are deprived of food usually die in two or three weeks.

During hot weather the beetles may be seen in slow flight about infested rooms. The usual mode of progression, however, is by rapid running. When roughly handled they feign death for a short time; as a rule they are negatively phototropic and quite wild, but those which are confined and exposed to light during the day and fed frequently become increasingly tame. On being held in the fingers or forceps a strong, very disagreeable, but transient, odor is emitted.

A photograph of several ham beetles is shown in Plate 1, A.

[3] The writers are indebted to Edward A. Chapin for the foregoing technical description.

[4] A similar service in stored products by a clerid has been reported by G. A. Runner (*26, p. 35*), who stated that *Thaneroclerus girodi* Chevrolat apparently at times causes the complete disappearance of *Lasioderma serricorne* Fab. from boxes of infested cigars.

Necrobia rufipes

A.—Adults. ×4
B.—Eggs laid on cardboard. (Showing the edge of a millimeter scale)
C.—Full-grown larvae. ×3
D.—Larva in cell made between plug of black cotton and side of vial
E.—Pupal cell on canvas sack, opened to show pupa and cast prepupal skin
F.—Pupae

THE EGG

The egg of *Necrobia rufipes* is about 1 mm. in length and 0.25 mm. wide, tapered, and roundly pointed at both ends and slightly curved in outline. It is smooth, shining, translucent, and is glued in place. As shown in Plate 1, B, the eggs are usually deposited in clusters. Those laid by old females often partially collapse laterally within a few hours after being deposited, and such shrunken eggs do not hatch.

Toward the end of the incubation period (four or five days in length during warm weather) the four eyespots of the embryo become visible, followed by pigmentation of the tips of the mandibles. Shortly after hatching, and in some cases before the escape of the larva, the caudal plate, head capsule, and prothoracic shield assume their final color.

HATCHING

The struggles of the hatching larva cause the posterior extremity to move about, with the result that the tubercles on the caudal plate tear the eggshell open at one end, the mandibles accomplishing the same at the anterior end. Thus the eggshell is usually torn open at both ends before the larva leaves it, and the larva often remains in the shell as in a short tunnel for several hours, feeding on the shell.

THE LARVA

TECHNICAL DESCRIPTION (*4, p. 597–599*)

Total length of body, about 10 mm.; extreme width, about 2 mm.; fifth to seventh abdominal segments widest; anterior width of prothorax one-half the width of the seventh abdominal segment; extreme thickness, 1½ mm.; seventh abdominal segment thickest. Corneous parts shiny, brown ocher; delicately chitinized parts shiny, pale clay yellow; membranous parts of thorax and abdomen dorsally mauve or lilac with white muscle attachments, ventrally whitish with bluish pattern. Frons rugose, anteriorly on each side of middle line with a shallow deepening. Labrum three times as wide as long; width about one-third the length of frons. Mandibles half as long as frons; length to width as 4 : 2; retinaculum and tooth same size, well developed, and rather obtuse. Two short mandibular setae. Prothoracic shield two-thirds as long as wide, with parallel sides. Both mesothorax and metathorax are about as long as prothorax, surpassing it one-third or more in width; metathorax a trifle wider than mesothorax; mesothoracic and metathoracic dorsal plates present, small, and about the same size. Basal plate of cerci a trifle wider than the prothoracic shield, length to width as 2 : 3. Cerci one-third the length of basal plate, upward curved, diverging about 60°.

LARVAL BEHAVIOR

The delicate, wrinkled, hairy larva after leaving the shell moves about but little for awhile, confining its activities as it gains strength to feeding on the unhatched eggs in the near vicinity and eating the shells of empty eggs. The shells are usually almost wholly consumed. New-laid eggs of the ham beetle, exposed to very young larvae which were also provided with dead skipper maggots, were all eaten. Eggs of *Dermestes vulpinus* Fab. were also eaten.

The postembryonic larvae are repelled by light, and, for the first day or so, prefer to spend most of their time hidden beneath some object, even when food is provided and light excluded.

The rearing of individual larvae (Table VII) was found to be practically impossible when fat bacon or skipper larvae were used as the only food. By feeding the postembryonic larvae with eggs of the ham beetle for 8 or 10 days after hatching, then giving both eggs and crushed skippers for 4 days and crushed skippers thereafter, single larvae were easily reared. To prevent the very small larvae from escaping or becoming entangled in cotton, the best container was found to be No. 11 veterinary capsules, the food being placed between two pieces of cardboard. The larvae thus reared reached a good size and molted two or three times before pupating. First skins measured 0.08 mm. between the tips of the caudal tubercles, second skins 0.25 mm., and third skins 0.5 mm. The molting skin splits over the thorax, the head is withdrawn from the head capsule and extruded through this opening, and the insect crawls out of the skin. There is much variation between the dimensions of the smallest and largest full-grown larvae, the latter being 100 per cent larger than the former.

Larvae of all instars are repelled by light. Well-grown larvae are able to crawl rapidly and to kill migrant skipper larvae. In their efforts to escape the maggots throw their attackers about with considerable force, but the latter seldom release their jaws until the maggots have become helpless. When touched, ham-beetle larvae protect themselves by thrashing about, bending their extremities together first on one side and then on the other. The full-grown larva is shown in Plate 1, C.

PUPAL CELL OR COCOON

Following the completion of feeding, full-grown larvae infesting smoked meat migrate from the greasy material in which they develop and seek a dark, dry spot in which to build the cocoon. At this time the larvae will not usually eat if skippers are offered to them.

The cocoon (pl. 1, D, E; pl. 2, D) may be completed within 24 hours, and is formed by filling in the open boundaries of the crevice chosen for pupation with a wall of white substance which is vomited at will from the mouth of the larva in frothy droplets. Each droplet appears only after the larva has chosen the location for the next unit of the wall, and it hardens into a vesicular mass immediately it is put in place.

During the process of cell building the larva is usually curled in the cell, although sometimes the inclosure is large enough to allow it to extend its length. At times cocoons are broken into by adults and the occupants devoured. It sometimes happens that two larvae inclose themselves in a common cell. On one occasion a larva was observed to cease construction work and to remain motionless, apparently as a means of protection, for several minutes while another larva was crawling on the outside of the cocoon. Frequently, when collecting immature material for experimental use, the writers secured numbers of larvae by opening cells, and many of these were able to resume normal activity promptly and to secrete without further feeding sufficient froth to construct another complete pupal chamber.

HE PREPUPA AND PUPA

Several days after the cocoon is completed the larva contracts in length, the body consequently becoming more robust; the head assumes a fixed attitude at right angles to the body axis, and the insect becomes a prepupa. The last larval skin is next cast and the pupa appears in the cell.

The pupa is restricted in movement to wriggling of the abdomen, to the tip of which the shriveled cast skin of the larva usually adheres. Unprotected pupae are readily devoured by adults. Pupation occasionally takes place without the protection of a cell. The pupa is illustrated in Plate 1, F.

DISTRIBUTION

In 1804, Latreille (*19, p. 156*) gave the distribution of the species as southern France and Italy. Stephens in 1830 (*32, p. 327–328*) reported it rare about London, though rather abundant in certain years. According to Curtis (*6, pl. 350*) the range of the insect was extensive, including southern France and Africa, although in Britain it was rare. Sharp (*30, p. 254*) stated that it is one of our most cosmopolitan species. Houlbert and Bétis (*15, p. 16*) gave its distribution in Brittany as widespread, but quite rare.

According to Lintner (*20*), the insect was introduced into the United States, and Froggatt (*9, p. 169*) believed the same to be true with respect to Australia.

Reports of injury to smoked meats have been received by the Bureau of Entomology from Texas, Tennessee, Georgia, Virginia, the District of Columbia, Maryland, and Pennsylvania, as shown by Figure 1.

In general, it may be said that *Necrobia rufipes* is a cosmopolitan insect. It is a species commonly

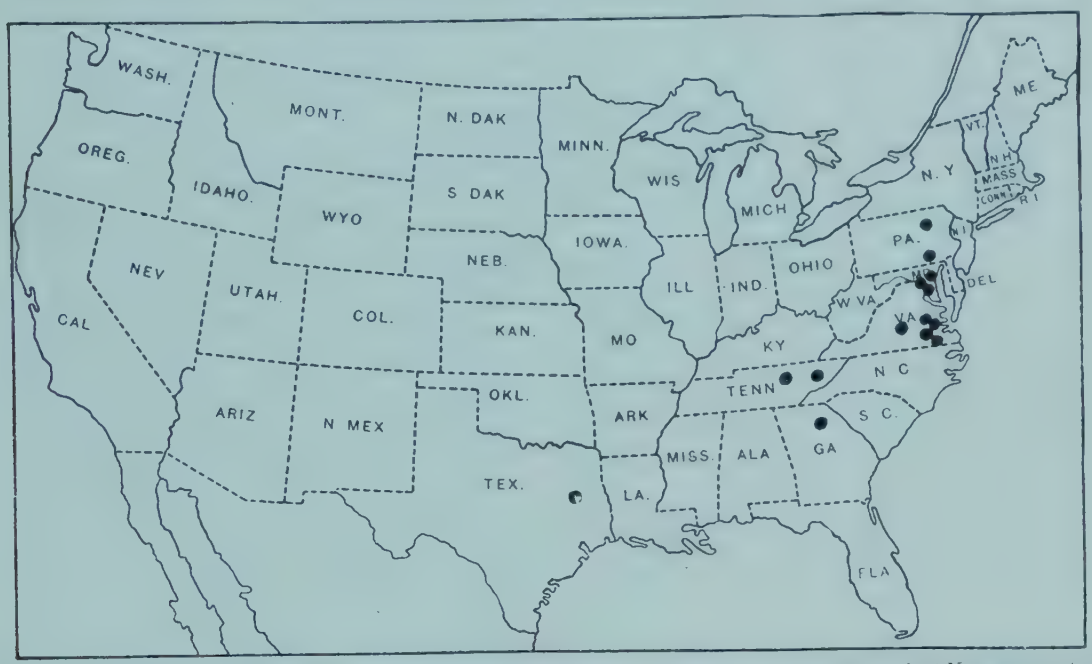


FIG. 1.—Map showing localities from which complaints of damage by the ham beetle (*Necrobia rufipes*) to smoked meats have been received by the Bureau of Entomology. Records of infested cargoes landed at various ports are not included

brought into our ports in cargoes. Records in the Bureau of Entomology show that it has arrived in cargoes in fish "guano" and bone meal from Honolulu, in garlic from New Zealand (probably having developed in other material), in coconuts from Manila, in bones from Argentina, in copra from the Philippines, in palm-nut kernels from British West Africa and Liberia, in herring and whale "guano" from Scandinavia, in dried egg yolk from China, in coconut palm from Ceylon, and in rattan from Japan. In bone storages it is frequently found in company with a closely related but economically unimportant species, *Necrobia ruficollis* Fab.

In the United States, as shown in Figure 1, reports indicate that most of the injury occurs in the Middle Atlantic States and that it is particularly marked in Virginia. Possibly this is due in a measure to the long storage of stocks of "Virginia" hams, which are not considered prime until they are about a year old.

SUBSTANCES INJURED

The first record of injury to human food by *Necrobia rufipes* seems to be that of Glover (*13, p. 97–98*), who reported it on cheese in Maryland. Riley (*24*) gave the earliest account of extensive injury to cured meats, concluding that attacks occur particularly to hams injured by overheating or by exposure to sun and rain. The species, he believed, is attracted by the fatty slime on hams.

Our chief concern in this country is with the infestation of smoked pork, other materials being attacked only on rare occasions.

The following list summarizes the known foods of this insect. The "substances infested but not fed upon" are sought by the migrating, full-fed larvae as suitable materials in which to pupate. Baled cotton and wool are sometimes badly matted with the pupal cells of the insect when these goods are carried in the same ship with bones.

Foods of the larvae or adults of the ham beetle, as recorded from literature, include: Cheese (*25, p. 226; 31, p. 266*), hams, bones (*34, p. 161; 31, p. 266*), fish (*25, p. 226; 31, p. 266*), drying carrion (*31, p. 266*), copra (*10, p. 26; 2*), hides (*5*), salt fish (*7*), bacon, dried egg (*29*), dried figs (*17*), and Egyptian mummies.[5] Bureau of Entomology records include: Dried egg yolk, hams, cheese, fish "guano"[6], bone meal, bacon, copra, bones, palm-nut kernels, and herring and whale "guano."[6] Substances infested but not fed upon, as recorded in published accounts, include: Silk (*28, p. 426*), baled cotton (*21*), and woolen tops (*11*); rattan and salt are given in the files of the bureau. A number of other references to literature, for the most part dealing with infestations of copra, are not included.

NATURE OF INJURY

Both larvae and adults feed upon smoked meats, the latter superficially. The larvae at first burrow beneath the hide, later extending their feeding deeper into the meat, chiefly in the fat portions (pl. 2, C). Frass is extruded from the burrows; this is shown in Plate 2, F. A piece of meat which had been seriously damaged on the flesh side is illustrated in Plate 2, A, and the work of the larvae in perforating a grease-soaked paper wrapping is shown in Plate 2, E. Plate 2, B shows the work of this species in old cheese.

BIOLOGICAL INFORMATION RECORDED BY OTHER WRITERS

The life history and habits of *Necrobia rufipes* have received little attention. Riley (*24*) stated that hibernation takes place solely as the larva and that no adults emerge before the first of May. On the other hand, Fay (*8, p. 197*) listed it among insects secured in winter collections.

[5] See reference to Hope on *Necrobia mumiarum*, under "Synonymy."
[6] Refuse used as fertilizer.

EXPLANATORY LEGEND FOR PLATE 2

Necrobia rufipes

A.—Longitudinal section of an old infested ham. In this case most of the feeding has been on the inside and has extended toward the skin side. Burrows may be seen penetrating the solid fat. Extensive feeding took place under the skin at the shank end, some of which is shown in the photograph

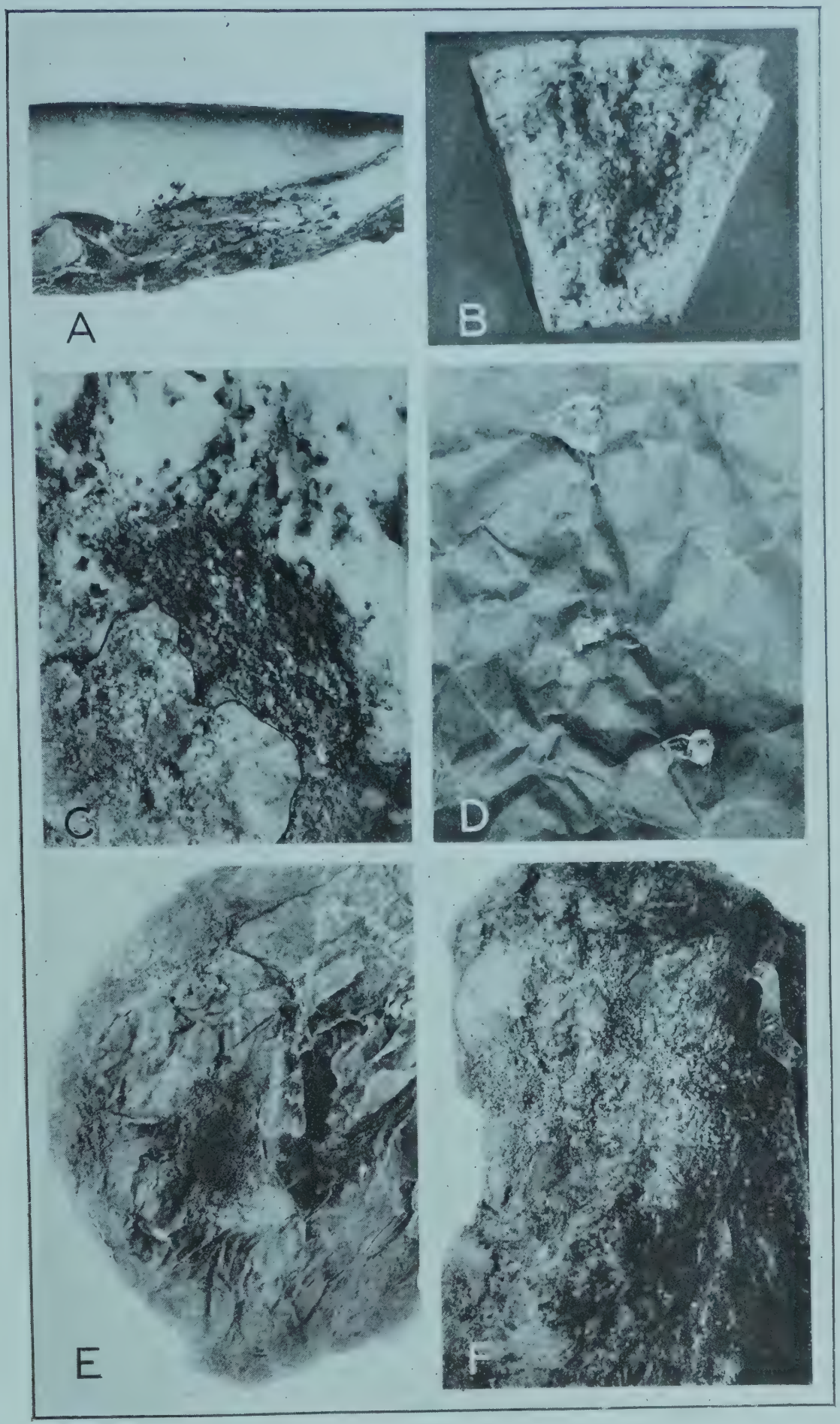
B.—Injured cheese. Several pupal cells are present in the excavated area. The dark spots are the excrement of the adult beetles. In this case the beetles and their larvae superseded an infestation of the cheese skipper (*Piophila casei*)

C.—Feeding burrows of larvae in the fat of an old ham. The cut surface, to the right of the black ink line, slants down into the tissues

D.—Pupal cells formed in creases of paper wrapper on ham. Cells torn open when paper was flattened out

E.—An old ham which had been wrapped for several months. The inner paper had become grease-soaked and the larvae penetrated it in the course of their feeding

F.—An old shoulder infested with larvae, showing the mealy frass from their burrows which has accumulated on the surface



(For explanatory legend see p. 850)

The beetles appear in May and June, according to Howard (*16, p. 105–107*), and never seem to oviposit except where the meat is more or less exposed. He also stated that E. A. Schwarz found adults in midwinter at Detroit, Mich., and Cambridge, Mass.

Herrick (*14, p. 278*) reported that pupation takes place in the more fibrous parts of ham, or sometimes in a rear-by beam. The globules of white substance which form the cocoons are emitted from the mouth of the larva.

Of the biology of the ham beetle as a copra pest, Dupont (*7*) wrote that "it is supposed to be a purely predacious insect, feeding on dipterous and microlepidopterous larvae," but it was not known whether or not it attacks the larvae of *Oryzaephilus surinamensis* L. (the saw-toothed grain beetle), which are also abundant in copra stores.

LIFE-HISTORY INFORMATION

OVIPOSITION

The act of oviposition has never been observed by the writers; presumably the eggs are deposited when the insects are in darkness. Owing to their carnivorous appetites the beetles devour eggs which are exposed, and the females usually deposit them in crevices inaccessible to the mandibles of roving adults.

Following unsatisfactory oviposition trials with pieces of stale bacon attached to strips of cardboard, Petri dishes were used in which the only suitable place for oviposition was between small squares of dark-colored cardboard held together with a paper clip. In dishes which were greasy throughout, oviposition was sparse until clean dishes and dry cardboard were provided. It

TABLE I.—*Incubation period of Necrobia rufipes, 1922*

Date eggs were laid [a]	Hatching began	Minimum incubation period	Temperatures during each period [b]			Date eggs were laid [a]	Hatching began	Minimum incubation period	Temperatures during each period [b]		
			Max.	Av.	Min.				Max.	Av.	Min.
		Days	°*F.*	°*F.*	°*F.*			*Days*	°*F.*	°*F.*	°*F.*
Mar. 28	Apr. 5	8	79	66	53	Sept. 24	Oct. 2	8	75	68	60
May 9	May 15	6	82	74	70	25	Oct. 3	8	75	68	60
11	May 16	5	82	75	70	26	Oct. 4	8	74	68	60
12	May 17	5	77	74	69	27	Oct. 5	8	77	69	60
15	July 19	4	82	77	69	28	---do----	7	77	70	66
20	July 24	4	84	79	72	29	Oct. 6	7	79	70	65
21	July 25	4	89	81	72	30	---do----	6	79	71	65
25	July 29	4	84	78	71	Oct. 1	Oct. 7	6	79	72	65
Aug. 16	Aug. 20	4	88	81	73	2	---do----	5	79	72	65
19	Aug. 25	6	83	75	66	3	Oct. 8	5	79	73	67
20	---do----	5	80	74	66	6	Oct. 11	5	78	73	67
21	Aug. 26	5	80	74	66	9	Oct. 17	8	77	68	59
23	Aug. 28	5	81	77	68	10	Oct. 18	8	77	68	59
24	Aug. 29	5	81	76	69	11	Oct. 20	9	77	67	59
25	Aug. 30	5	81	76	69	12	Oct. 21	9	77	67	59
26	Aug. 31	5	81	75	69	13	---do----	8	77	67	59
27	Sept. 1	5	80	75	69	14	Oct. 23	9	77	67	58
28	Sept. 2	5	80	74	68	15	Oct. 24	9	75	67	58
29	Sept. 4	6	80	75	68	16	Oct. 26	10	77	67	58
30	---do----	5	80	75	68	17	Oct. 27	10	77	67	58
31	Sept. 5	5	82	76	68	18	Oct. 28	10	77	67	58
Sept. 1	---do----	4	82	76	68	19	Oct. 30	11	77	66	58
2	Sept. 6	4	82	77	72	20	Oct. 31	11	77	65	56
3	Sept. 7	4	84	78	73	25	Nov. 5	11	77	66	56
4	Sept. 8	4	85	79	75	26	---do----	10	77	66	56
5	Sept. 9	4	85	79	75	27	Nov. 7	11	77	66	56
6	Sept. 10	4	85	78	75	28	---do----	10	77	66	56
7	Sept. 11	4	85	78	75	Nov. 3	Nov. 14	11	77	67	58
8	Sept. 12	4	83	78	75	4	---do----	10	77	67	58
9	Sept. 13	4	83	78	75	6	Nov. 16	10	77	67	58
10	Sept. 14	4	83	76	70	7	Nov. 17	10	77	67	58
11	Sept. 15	4	83	76	70	8	Nov. 18	10	77	67	58
12	Sept. 16	4	81	75	70	12	Nov. 22	10	76	66	56
13	Sept. 19	6	81	72	58	16	Nov. 29	13	77	64	49
14	Sept. 20	6	81	71	58	17	Dec. 2	15	79	64	49
15	Sept. 21	6	81	70	58	24	Dec. 7	13	79	64	49
16	Sept. 23	7	80	70	58	28	Dec. 9	11	79	65	53
20	Sept. 28	8	76	68	60	29	Dec. 13	14	79	66	53
21	Sept. 29	8	76	68	60	Dec. 1	Dec. 14	13	79	66	53
22	Sept. 30	8	76	68	60	2	---do----	12	79	66	53
23	Oct. 2	9	76	69	60						

[a] Number of eggs laid each day varied from about 25 to 100.
[b] Average temperatures computed from thermograph readings every 2 hours.

is concluded that a close, dry crevice is preferred by the laying females.

INCUBATION PERIOD

At temperatures of 70° to 85° F. the usual incubation period is four or five days. In early winter, when temperatures became low in the laboratory at night, incubation periods of two weeks were recorded. Batches of eggs hatched with uniformity as regards time; the last eggs to hatch produced larvae about one day after hatching began, in warm weather. Table I shows incubation periods observed in 1922 at Washington, D. C.

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The results of oviposition in vials containing stale bacon as food for the adults show an average of 137 eggs and a maximum of 312, which does not indicate that the ham beetle is unusually prolific. When the beetles are fed on maggots, however (see Table IV), a far greater capacity for increase is indicated, and it is instructive to compare Table IV with Table II.

The eggs laid by females fed with maggots of the skipper fly in several cases totaled over 1,000 and were obtained in the following manner. Pairs were mated shortly after emergence from the cocoon and were fed daily, except Sunday, with three migrant larvae of *Piophila casei*, maggots in excess of three being usually uneaten. The results of the oviposition of some of the pairs are given in Table III, which includes 20 of the best records from a series of 76 pairs. The discontinuance of another series of 32 pairs, mated in March, 1923, became necessary after six of the females had laid from 444 to 1,110 eggs each.

TABLE II.—*Oviposition and longevity of Necrobia rufipes, 1922*

[Food: Stale fat bacon]

Pair No.[a]	Total eggs laid	Male emerged	Male longevity	Female emerged	Female longevity	Pair mated	Mating to oviposition	Duration of oviposition	End of oviposition to death of female	Laboratory temperatures (° F.)			
										Month	Maximum daily mean	Average daily mean	Minimum daily mean
			Days		*Days*		*Days*	*Days*	*Days*				
1	312	Feb. 23	135	Feb. 23	-------	Feb. 27	-------	-------	-------	Feb	71	67	59
2	10	Mar. 4	-------	Mar. 4	49	Mar. 6	24	6	17	Mar	72	67	59
3	155	Mar. 5	-------	do	-------	do	-------	-------	-------	Apr	80	69	58
4	140	Mar. 6	186	Mar. 3	169	Mar. 8	-------	-------	-------	May	79	73	67
5	78	Mar. 7	160	Mar. 7	160	do	22	107	30	June	86	79	70
6	239	Mar. 9	104	Mar. 9	142	Mar. 10	14	97	30	July	87	79	71
7	68	do	158	do	175	do	...	...	...	Aug	83	76	71
8	109	do	163	do	183	do	26	104	52	Sept	81	73	62
9	38	Mar. 10	85	Mar. 10	90	do	14	35	41				
10	111	Mar. 11	118	Mar. 11	161	Mar. 14	16	111	31				
11	233	do	-------	do	94	do	6	85	0				
12	192	Mar. 13	87	Mar. 10	137	do	6	72	55				
13	94	Mar. 15	-------	Mar. 15	-------	Mar. 18	24	88	-------				
Total	1,779	----------	1,196	----------	1,360	----------	152	705	256				
Average	137	----------	133	----------	136	----------	17	78	32				

[a] These pairs were used in preliminary experiments, but the results clearly show that an exclusive diet of smoked pork is not the most favorable food for adults kept in close confinement. Compare with Table IV.

TABLE III.—*Oviposition of Necrobia rufipes*

[Food: Larvae of *Piophila casei* L. Legend: EM, emerged and mated]

Date of oviposition	Eggs laid by female of pair No.																				Daily mean temperature
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1922																					°F.
Sept. 1	EM																				73
6		EM	EM	EM																	80
8	11	18		10																	76
9		31	6	37																	77
10		20	54	25																	78
11		3	38	34	EM																79
12		10	7	32		EM															77
13			36																		73
14			10	14																	74
15	1		16	15																	77
16		13	8	25			EM	EM	EM	EM											76
17			25			17															71
18						12					EM	EM									65
19													EM	EM							68
20		10	17				5								EM						69
21				6												EM					71
22			16	13							22										69
23		26					6				6										70
24		22	23	24		29	3	6			33		12								73
25		44	25			3	6				15		24								67
26						12															62
27							6	1					3								65
28	12	32				31	22				12		19								68
29		8																			70
30		20	17			8	16				5										70
Oct. 1			17								14										69
2	8					22															69
3		16	8			10					14					5					70
4			26			18					11		12								73
5	7	21	9	6		14	5				23										74
6		21	21			3	15	12			13			5			EM				74
7		19	8	20		23	19			14	16			13		2					73
8	6	27	12					10		8	25	10				11					74
9	3	17	23			21				2	8					9					71
10	5	19				20	2	18						6							73
11	2		13	2		16	9			2	16		5	6		16					67
12		33	10				12				14			11							64
13						4															65
14			12	9		19	7	14			6		29	9							68
15				5							23					27					68
16		19	23			31					20						EM				69
17		16	5							2	9			3							70
18		10	11											17				13			68
19		17											17					28			67
20			13			22								13	5	19		20	EM		66
21											10							17		EM	65
22						17															60
23				5		15	3				6			4		7		18			65
24		44	14				6				8							8			68
25			8			3				8		4	23	19			8	13			66
26			4			3					27			6				24			67
27																		6			65
28	7		23			25						12				7	34	3	9		67
29													6	5							62
30	5					8															65
31	8																	19			64
Nov. 1			8			4							14	5		8			9		68
2	9	11						8			8	5		8		8		7	7		68
3	5		21							9	11			5	6	16		23			68
4			18						13		17			16	14				8		68
5			4									20	9			10		13	15		69
6	3	20	11			31					12					3		9			68
7	8		9											14							69
8			23			28			1	31				21				28			66
9			26			24			14					10	5			26	9		67
10	9		15			16			5	14	18		10	16	11	20		10	14		66
11		24	24	10					6		5		17	12				6			67
12			8			30				9											67
13	23		28								6	8						15	11		62
14			9			17				14	18		25	18		8		16	24		67
15						17					6					3					67
16	13		28	4						6	8			13				5			69
17	12		3			33				10	8			6				21			67
18	16		21				7				13			13					10		67
19	6		18			27								9					7		67
20						12															63
21	7					12										14		55			64
22	14					10					7		10	14	5						65
23	9												11								63
																					64

TABLE III.—*Oviposition of Necrobia rufipes*—Continued

Date of oviposition		Eggs laid by female of pair No.																			Daily mean temperature
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	°F.
	24			9								13										66
	25											7								9		66
	27			18											14							63
	28	10												21						14		65
	29			26							13						9					67
Dec.	1										17	4								6		66
	2			27							9	11										68
	5	10		38								5			(a)				26			69
	6													4			4					65
	7	19		12			5				5											65
	8			22							5		5									67
	9	10																				71
	11	19					23				11	6	12	16					13	19		68
	12	17					17												15			68
	13						13				20		24	3				13	4			67
	14									7										5		67
	15	5					27							20		12				16		69
	16	21		28							19	23	27				16		22	15		69
	18			25			12				15	5		22					20	15		66
	20																11					68
	21	16		24			18			6	4								18			68
	23			7							13								16			66
	26	28		9			30				24			22			22	3	41			67
	28	5		3			9			2								5	14			69
	30	22					19			7	21	4	13		14				20	31		69
1923																						
Jan.	2	32					14							25	5		6	19	30			71
	3				5		3															68
	5						17								10			15		6		69
	8												20		20			20				67
	9						16												15			69
	10						15				7		18	21				16	15			66
	11	17	2	1			9								19				9	20		66
	12												25				17	29	17			67
	13										11									8		68
	14						24							20								62
	15	21					26						13		19			21		6		66
	16	17									16		15			5		18	12			67
	17	6										(b)			8	12				20		66
	18	8									23			16	15				14			69
	19	6					24			6	3							39		26		72
	20						8												8			70
	22	14						10		6			20	13				22				67
	23	18					23				13		6		14							70
	25		10				9				12		17		37		6	8	12			72
	26						6				8		8	13						23		72
	27										15											71
	29										11							12	22	17		68
	30	8											14						6	8		68
	31	11					10				18					10			16			69
Feb.	1	14	5													4		7				70
	2						7				39		19	14	5		11	24				69
	3													12	9	4		14	21			71
	5						6				19											66
	6												10					18				69
	7	7					6	4								3			11			71
	8										8				9			4	15	8		70
	9										8		21			10						70
	10												17					2	19	5		67
	12	17	42				3						45	22	12							66
	13					5		39			15		14		10				5		11	70
	14	7	5								17		48			23						68
	15							40		5					8						11	69
	16		20				5			5			33	16	6				7			65
	17	23				7									5	20	9				8	69
	18							19														64
	19		29					7			12		67			12					3	68
	20	14	5				5	4									8		5	8		70
	21	18								4			25		9	16						67
	22							28			23									27		66
	23	20					6						12		20	29						67
	24									31								(c)				64
	25																39			44		68
	26							15					(c)			5				16		70
	27	9						27			3				15	17	6		5			70
	28	6																		7		70

[a] Male escaped. [b] Female died. [c] Male died.

TABLE III.—*Oviposition of Necrobia rufipes*—Continued

Date of oviposition	Eggs laid by female of pair No.																			Daily mean temperature
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1923																					°F.
Mar. 1	6												6						13		68
2												5	6		26				8		68
3	7	13			9							13		6		26					73
4	14																				73
5					6					26			18	6							71
6		40													13				25		69
7		34			5		7		20				11		24						71
8							3		5					6	7				5		69
9	10						7		32			4							7	6	70
10										25											68
11	17																				62
12	6	32					17								16	21			14		71
13					19		19		23			6							31		74
14					7				23							14			9		71
15	12	50			4				14	20		13			18				12	7	69
16							12					21			8	2			4		68
17		39			5				21				13			5			11	6	68
18									25												61
19	7	47			14							45	19		36					28	66
20							5													6	68
21									17												70
22	2									8					44				22	18	73
23							19														75
24		67							38	34		16			7				43	28	74
25				[a]					8	15		4			7				[a]		66
27		59													19	20				17	68
28									6												71
29										19		6	18								69
30									24												69
31																					
Apr. 1										3										2	71
2		0			15										42						56
3		4			1		8					8				8					71
4					15											9				12	72
5	5																				74
6	4														50						73
7		54					23		5			26								6	73
9									24												73
10		54			11				7	6		3	9		9	20				9	68
11	15								3												69
12									14	19			18							24	69
13									19												71
14	11								5	21			24			8					72
16					28		7		26	10										57	69
17																8					68
18	10								16			16			9						70
20			[b]		32					10			20								71
21	1				10				28				15							32	73
23		24	[a]							30			8		45						75
24					19			2													74
25					12		5	5							8	12					65
26							[a]	5					17							18	71
27												14									71
28		6					7						16		45	28		[b]		30	71
30		52							27	25						16				47	74
May 1	[a]				32										11					5	68
2	15				14				10											13	70
3		26			27																71
5					7			4		19					11	22				28	71
7						[b]		7		10		11			53					30	74
8					6											6					73
9					20				4	4						16					73
10										3										7	71
12						[a]			[a]				[a]							25	66
14															7	27					71
15																				15	72
16					9				7	9										8	74
17					12					15						34				24	78
18					24											5					76
19								14		12					4	22					73
20											[a]										76
21		[b]						34		8						19					76
22															25					23	76
23					11			12								10				20	75
24					15			22		20						35				11	71
25					2					12					26	19				5	72
26					17			12		9					12					6	73
28					6			22	13						7						74
29								11							26	40				36	80
31			[b]		12										11	7				7	82
																12					75

[a] Male died.

[b] Female died.

TABLE III.—*Oviposition of Necrobia rufipes*—Continued

Date of oviposition	Eggs laid by female of pair No.																			Daily mean temperature
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1923																					
June 1												[a]									77
2					11										27	42				18	84
4								17		11										15	85
6					20			7		23				[a]	11	22					85
7					43			7		5					[b]	24					84
8					4					11						23				27	81
9					22			24							43	31					75
11					30			26							22	48				29	76
12								16													72
13																24					71
14					11										10	9					72
15					40			11								57				51	78
16					12			10							32	12					79
18					18			24							33	64				34	83
19					10										10	6					84
20					17			22	[a]	[a]						44					87
21					23			15								24					89
23					27			11								51					85
24																				[a]	86
25					26			23								37					88
26					13			11								13					90
28					8			30								33					79
29					13																74
July 2					14		[a]	[b]								28					73
3					40																75
4					[b]			10													78
5																35					81
6								17													82
8					20			6													79
10					12											16					79
11					12			15								45					81
12					16																80
13								17								44					79
16					42			19								43	[a]				82
18					11			20								11					79
20					13			20					[a]			26					81
21					4											8					85
24					10			27								45					73
25																8					76
26								19								16					76
27					22			4								29					77
28																23					80
30								48								18					80
Aug. 1								30													69
2								17								20					72
3					15			27								16					80
4					11			27								38					81
6					26			108								53					84
7								31								13					85
8								37								41					86
9								30								20					78
11								43								32					78
13					5			40								33					79
14								12								14					76
15								8													80
16					5			24								16					77
17					9			28								18					72
18								15													71
20					25			38								19					78
21					7											5					78
22								29								4					75
23					11			25													69
25								19													73
27					6			28													75
30					5			17													75
Sept. 1					22																76
4								42													80
6					8			20													72
10																[b]					69
18								43													67
24					[a]											[a]					78
26								51													76
28								12													75
Oct. 30								[a]													
Nov. 13																			[a]		
20										[b]											
Total	846	1316	1087	301	1197	1088	481	1497	583	1029	576	854	744	598	1042	2131	351	876	706	823	

Average eggs per female 906. Date of death of female No. 15 not recorded.

[a] Female died. [b] Male died.

In Table III the conspicuous features are the large numbers of eggs laid by several of the females, the length of the records, the marked differences in fecundity of the females, and the long rest periods during which no eggs were laid. As the present report appears to be the first detailed account of the biology of a representative of the large family Cleridae, there is no opportunity for comparison of the life history of *Necrobia rufipes* with an allied form.

As shown by Table III, mated females continued to lay eggs for several months after having been deprived of the male through its death or escape. The male of pair No. 14 escaped December 5; eggs laid by the female January 18 and March 5 produced larvae. As previously noted, eggs laid by old females not infrequently collapse and fail to hatch. This occurred, for example, with the eggs of pair No. 2, which were laid April 23 and May 3, and the eggs of pair No. 9, laid on May 2. The records detailed in Table III are summarized in Table IV.

TABLE IV.—*Oviposition and longevity of Necrobia rufipes, 1922 and 1923* [a]

[Food: Larvae of *Piophila casei*]

Pair No.	Male and female emerged and mated	Longevity of male	Longevity of female	Duration of period, mating to oviposition	Duration of period of oviposition	Duration of period, end of oviposition to death of female	Total number of eggs laid
	1922	*Days*	*Days*	*Days*	*Days*	*Days*	
1	Sept. 1	242	(b)	7	236	(b)	846
2	Sept. 6	375	258	2	237	19	1,316
3	---do----	229	226	3	124	99	1,087
4	---do----	201	267	2	119	146	301
5	Sept. 11	296	378	155	205	18	1,197
6	Sept. 12	242	237	5	159	73	1,088
7	Sept. 16	222	289	4	221	64	481
8	---do----	289	409	8	369	32	1,497
9	---do----	238	277	58	196	23	583
10	---do----	430	277	28	237	12	1,029
11	Sept. 18	234	121	4	99	18	576
12	---do----	161	256	20	211	25	854
13	Sept. 19	235	304	6	215	83	744
14	---do----	(b)	260	17	153	90	598
15	Sept. 20	229	-----	30	-----	-----	1,042
16	Sept. 21	354	368	12	323	33	2,131
17	Oct. 6	131	283	22	43	218	351
18	Oct. 16	325	194	2	132	60	876
19	Oct. 20	157	389	8	147	234	706
20	Oct. 21	248	246	113	127	6	823

[a] This table is a summary of the records detailed in Table III. Compare with Table II.
[b] Escaped.

DEVELOPMENTAL PERIOD

In the first rearing trials larvae were provided with what appeared to be the preferred food—stale bacon. This was heated to about 120° F. for a day or two in order to extract some of the low-melting-point fat, which was so abundant in unheated pieces of bacon that the larvae were often smothered in it. Although larvae usually become large and vigorous when they develop in whole hams, shoulders, or sides of bacon, the mortality in vials containing small pieces of the same meat was high and development slow, frequently resulting in dwarfed individuals. The results of the rearing experiments in which stale fat bacon was used are assembled in Table V.

Trials shown in Table V were preliminary, but the results, compared with those given in Tables VI and VII, show that an exclusive diet of smoked pork is not the most favorable food for larvae reared in close confinement.

In some of the rearing work done subsequently to that shown in Table V the ham-beetle larvae were fed only skipper maggots. Newly hatched larvae were transferred to Petri dishes, 10 or 20 to a dish, where they were given crushed skippers until nearly full grown, when live skippers were provided. The mortality in these dishes was nearly always high. There was probably cannibalism, and the soft posterior proleg of the very young larvae often stuck to the smooth glass, resulting in their death by starvation.

Records of the development of larvae fed with skippers are given in Table VI. A comparison of this table with Table V shows the advantage to the insects, when closely confined as larvae, of a diet of maggots in the place of stale bacon.

In Table VI the shortest developmental period is shown to have been 30 days, including 17 days as a growing larva and 13 days within the cocoon as larva, prepupa, pupa, and adult. According to the results included in Table I the incubation period may be as short as 4 days; the minimum preoviposition period recorded (Table IV) is 2 days. The life cycle, therefore, is possibly as short as 36 days.

The details of the larval life are given in Table VII. In this set of experiments individual larvae were reared in large veterinary capsules, as explained previously under "Larval behavior." The larval stage is divided into three, sometimes four, instars.

PUPAL PERIOD

Details as to the duration of the pupal period and the prepupal period within the cocoon were secured both by opening cocoons and by examination of the insects in cells that had been built against the glass of the vials. In general, the prepupal stages (resting larva and prepupa) occupied about the same time as the pupal stage. Table VIII gives the results of these examinations.

OVERWINTERING

In an experiment made by the writers to determine the ability of full-grown larvae to survive 48° to 50° F. in a refrigerator, five out of seven lived about six months and one survived for seven months.

On December 21, 1922, an old shoulder infested with adults and various sizes of larvae was exposed to outdoor temperatures. The meat was kept in a fumigating box, protected from rain and snow, until March 24, 1923. After three months of winter temperatures, all adults and small and half-grown larvae were found to be dead, and many full-grown larvae were dead. Seven full-grown larvae survived, however, and eventually transformed into adults.

From these records, full-grown larvae appear best able to withstand such winter temperatures as obtain at Washington.

CONTROL SUGGESTIONS

Riley (*24*) recounted the control measures used by a concern in St. Louis. This firm dipped wrapped hams in a mixture of flour, water, a little glue, and chrome yellow, sometimes with "heavy spar" (barium sulphate) added. He suggested that a heavier canvas be used and applied before the first of May. Following the adoption of Riley's suggestions, losses were practically eliminated.

It should be observed that these suggestions were made in the days before artificial refrigeration was widespread, when hogs were necessarily slaughtered in the cool months. Practically all pork for use in summer had to be cured or smoked, or both, during

TABLE V.—*Development of Necrobia rufipes, 1922*

[Larval food: Stale fat bacon]

Date eggs were laid	Number of eggs in vial	First cocoon formed	Emergence began	Minimum period, egg deposition to cocoon formation	Minimum cocoon period [a]	Minimum period, egg deposition to adult emergence	Average daily mean temperature during egg to adult period
				Days	*Days*	*Days*	° F.
Mar. 22	28		Aug. 17			148	75
24	5		July 14			112	74
24	25		Sept. 3			163	75
24	21		July 31			129	75
26	33		July 11			107	74
30	33		July 9			101	74
30	11		Aug. 31			154	75
30	3	July 20	[b]	112		[b]	
Apr. 1	9		July 9			99	74
1	8		do			99	74
9	26	July 3	July 18	85	15	100	75
9	3	July 15	July 29	97	14	111	76
11	4	July 28	Aug 9	108	12	120	76
15	11	Aug. 15	Sept. 7	122	23	145	76
19	7	July 15	Aug. 4	87	20	107	76
19	11	July 24	Aug. 17	96	24	120	76
28	8	July 12	July 27	75	15	90	77
May 5	24	Aug. 5	Aug. 31	92	26	118	77
13	13	Aug. 3	Aug. 26	82	23	105	77
13	13	do	[b]	82		[b]	
16	10	July 20	Aug. 26	65	37	102	77
June 21	11	Aug. 3	do	43	23	66	78
Total				1,146	232	2,296	
Average				88	21	115	

[a] The figures in this column represent probable but not actually known minimum cocoon periods. In each vial several cocoons were formed in the cotton plugs, and the first adult to emerge did not necessarily come from the cocoon which was formed first.

[b] No emergence.

TABLE VI.—*Development of Necrobia rufipes, 1922* [a]

[Food: Mature larvae of *Piophila casei* L.]

Date egg hatched	Larval period	Cocoon period	Hatching to emergence	Date egg hatched	Larval period	Cocoon period	Hatching to emergence
	Days	*Days*	*Days*		*Days*	*Days*	*Days*
Sept. 5	28	13	41	Sept. 23	25	16	41
5	34	11	45	23	28	15	43
5	35	10	45	23	28	15	43
5	34	14	48	23	28	17	45
5	34	15	49	23	37	15	52
5	35	14	49	23	42	16	58
5	35	14	49	29	24	15	39
5	35	15	50	29	26	14	40
5	41	12	53	29	42	15	57
5	56	16	72	Oct. 2	28	14	42
5	80	19	99	2	28	15	43
12	30	14	44	2	28	17	45
12	30	14	44	3	23	18	41
12	30	15	45	3	41	26	67
12	30	16	46	3	41	29	70
12	36	13	49	3	55	18	73
12	36	13	49	4	26	15	41
12	36	14	50	4	30	17	47
12	36	15	51	4	30	18	48
12	37	14	51	4	48	16	64
12	36	15	51	4	35	52	87
12	36	15	51	6	38	16	54
12	36	15	51	6	41	18	59
12	36	16	52	6	46	16	62
12	36	16	52	6	41	21	62
12	36	16	52	6	46	16	62
12	37	15	52	6	46	17	63
12	36	16	52	6	48	16	64
12	39	13	52	6	46	18	64
12	36	17	53	6	48	18	66
12	39	15	54	6	46	20	66
12	41	15	56	6	48	19	67
12	43	14	57	6	48	20	68
12	44	14	58	6	48	20	68
12	45	17	62	6	48	21	69
12	48	16	64	6	52	19	71
12	48	16	64	6	52	22	74
12	59	16	75	6	52	29	81
12	59	18	77	7	45	16	61
12	65	16	81	7	45	16	61
20	28	13	41	7	45	16	61
20	28	13	41	7	45	17	62
20	28	17	45	7	45	18	63
20	35	19	54	7	47	16	63
20	40	14	54	7	47	18	65
20	54	16	70	7	47	18	65
21	25	11	36	7	45	24	69
21	25	12	37	8	19	17	36
21	25	13	38	11	19	15	34
21	25	14	39	11	38	7	45
21	32	17	49	11	28	24	52
21	32	18	50	11	49	20	69
23	17	13	30	11	49	22	71
23	17	13	30	12	26	15	41
23	23	8	31	15	22	14	36
23	19	13	32	15	39	15	54
23	23	12	35	15	37	17	54
23	23	12	35	15	39	19	58
23	19	16	35	15	47	21	68
23	23	13	36	15	43	29	72
23	23	15	38	15	54	19	73
23	23	15	38	17	35	17	52
23	23	15	38	18	40	22	62
23	25	14	39	20	47	22	69
23	23	16	39	26	36	27	63
23	28	12	40	26	43	20	63

[a] Larvae reared in Petri dishes. Sept. 5 to 26, exposed to laboratory temperatures, average daily mean 76° F.; Sept. 26 to Nov. 3, in incubator at 80° to 85° F; Nov. 3 to Dec. 8, temperatures not recorded but were usually between 70° and 80° F. All cocoons incubated at 80° to 85° F.

the season of low temperatures and stored for future consumption.

According to Perkins (*23, p. 126*) the larvae do not seem to be able to eat their way through any covering, and thoroughly wrapped hams are not therefore exposed to injury.

In the Philippines this species is controlled in copra by fumigation under a tarpaulin with carbon disulphide (*10*).

Herrick (*14, p. 280*) stated that the injured parts of infested meats can often be cut away.

Ashbrook, Anthony, and Lund (*3, p. 25–26*) have given an important control measure, which consists of removing the original string from smoked meats before wrapping them and tying a new string tightly around the outside of the package. This is necessary because it is impossible to make an insect-tight package if a string passes from the meat through the wrappings.

Ordinarily there appears to be slight danger of infestation of newly smoked pork products by ham beetles. If meats are wrapped promptly and tightly, there should be no trouble, but long-stored hams and sides of bacon that are hung up unwrapped or packed in crates accessible to the beetles are likely to become infested during warm weather.

The ham beetle is able to find an entrance to meat which is apparently tightly covered, and in view of the insect's ability to reach smoked meats protected by several overlapping layers of paper it is hardly to be wondered at that the theory of abiogenesis has been used to explain the presence of purple worms in these products.

Careful screening with fine wire cloth (30 meshes per inch), as recommended to exclude the cheese skipper, will also be effective against ham beetles.

Reconditioning by trimming off infested parts with a knife is sometimes resorted to, especially with bacon. The eradication of an infestation will be hastened if the meats and rooms in which they have been stored are fumigated with hydrocyanic-acid gas. The use of this gas for the fumigation of meats has been approved by the Bureau of Animal Industry, United States Department of Agriculture (*33*).

TABLE VII.—*Development of individual larvae of Necrobia rufipes*

[Food: Eggs of *N. rufipes* followed by larvae of *Piophila casei*]

Date egg hatched	Duration					Date egg hatched	Duration				
	First instar	Second instar	Third instar	Fourth instar	Larval stage		First instar	Second instar	Third instar	Fourth instar	Larval stage
1923	*Days*	*Days*	*Days*	*Days*	*Days*	1923	*Days*	*Days*	*Days*	*Days*	*Days*
May 13	8	6	10	13	37	May 15	9	6	10	----	25
13	10	6	13	----	29	15	7	8	16	----	31
14	7	8	15	----	30	16	6	8	19	----	33
14	11	4	10	11	36	16	6	8	8	----	22
14	7	8	8	10	33	16	6	8	8	11	33
14	7	8	8	16	39	16	6	8	19	----	33
14	9	6	10	8	33	16	6	6	10	----	22
14	9	6	21	----	36	16	6	8	19	----	33
14	9	6	21	----	36	16	6	8	8	----	22
14	9	8	19	----	36	16	8	6	22	----	36
15	7	8	8	13	36	16	8	6	16	----	30
15	7	6	24	----	37	16	6	8	10	----	24
15	7	8	10	----	25	16	8	8	14	----	30
15	7	8	10	----	25	16	6	8	10	12	36
15	9	6	22	----	37						

NOTE.—Mean temperatures (°F.): May 13, 73°; May 14, 72°; May 15, 74°; May 16, 78°; May 17, 76°; May 18, 73°; May 19, 76°; May 20, 76°; May 21, 76°; May 22, 75°; May 23, 71°; May 24, 72°; May 25, 73°; May 26, 74°; May 27, 78°; May 28, 80°; May 29, 82°; May 30, 77°; May 31, 75°; June 1, 77°; June 2, 84°; June 3, 86°; June 4, 85°; June 5, 87°; June 6, 85°; June 7, 84°; June 8, 81°; June 9, 75°; June 10, 76°; June 11, 75°; June 12, 72°; June 13, 71°; June 14, 72°; June 15, 78°; June 16, 79°; June 17, 80°; June 18, 83°; June 19, 84°; June 20, 87°; June 21, 89°; June 22, 89°.

SUMMARY

The ham beetle (*Necrobia rufipes* De G.) is widely distributed over the warmer parts of the world, frequently being found as an inhabitant of stores of bones and other inedible animal products. Among the islands of the Pacific it is a pest of copra. In parts of the United States sporadic injury, occasionally of considerable extent, is done to long-stored hams, shoulders, and bacon.

The adult is a shiny, green beetle 3.5 to 7 mm. long; the larva is purplish and about 10 mm. in length when full grown. Both imago and larva are

active and predatory; their habit of infesting smoked pork has probably been acquired rather recently. The larvae are responsible for most of the damage to infested meats. They bore holes in the meat, preferably burrowing into the fat parts. The adults feed chiefly on the surface. Pupation occurs within a white cocoon constructed with drops of froth emitted from the mouth of the larva.

The adult may live for more than 14 months, the female depositing as many as 2,100 eggs during that time. In warm weather the incubation period is four days or more. The preoviposition period is as brief as two days. The period from hatching of the egg to adult emergence may be as short as 30 days, including 17 days as growing larva and 13 days within the cocoon.

An important consideration in the prevention of injury by the ham beetle is the careful wrapping of meats. Probably the most effective method for the eradication of an infestation is a thorough fumigation with hydrocyanic acid gas.

TABLE VIII.—*Cocoon period of Necrobia rufipes, 1922*

Cocoon formed	Prepupal period in cocoon [a]	Pupal period	Cocoon period	Average temperature during cocoon period	Cocoon formed	Prepupal period in cocoon [a]	Pupal period	Cocoon period	Average temperature during cocoon period
	Days	*Days*	*Days*	*°F.*		*Days*	*Days*	*Days*	*°F.*
Jan. 17			42	67	Aug. 21	17	4	21	76
17			46	67	21	10	12	22	76
17			55	67	21	9	14	23	76
18			51	67	21	15	8	23	76
19			45	67	21	8	18	26	76
20			47	67	21	5	22	27	76
20			55	67	21	9	34	43	73
21			41	67	21	26	54	80	71
21			47	67	22	10	10	20	76
22			40	67	22	17	31	48	73
22			52	67	23	16	4	20	77
24			36	67	23	16	6	22	76
24			42	67	23	14	10	24	76
24			44	67	23	14	10	24	76
24			45	67	23	14	12	26	76
24			46	67	23	16	12	28	75
24			49	67	24	13	5	18	76
26			46	67	24	15	8	23	76
27			41	67	24	14	9	23	76
28			41	67	24	13	12	25	76
28			58	67	24	13	13	26	76
Feb. 4			46	66	24	15	15	30	75
Mar. 25			32	69	24	19	23	42	73
25	14	21	35	69	24	17	27	44	73
26	12	21	33	69	25	14	8	22	76
26	12	28	40	69	25	15	7	22	76
27	12	21	33	69	25	13	9	22	76
28	12	24	36	69	26	13	8	21	76
31	10	21	31	69	26	12	15	27	75
Apr. 1	10	22	32	69	26	15	13	28	75
3	9	24	33	70	28	9	10	19	76
9	9	21	30	70	28	9	13	22	76
Aug. 14	7	4	11	77	28	13	11	24	75
15	4	9	13	77	28	12	30	42	73
15	9	12	21	76	29	8	10	18	77
15	10	11	21	76	Sept. 1	10	8	18	76
15	14	8	22	76	2	6	8	14	77
16	6	11	17	76	2	8	22	30	73
16	9	12	21	76	2	9	23	32	73
16	13	9	22	76	5	6	9	15	75
16	12	11	23	77	5	6	21	27	72
17	11	5	16	76	5	6	21	27	72
17	11	9	20	76	6	14	13	27	72
17	11	9	20	76	14	22	12	34	70
19	8	11	19	76	14	17	19	36	70
19	8	14	22	76	16	15	15	30	70
19	17	7	24	76	16	14	18	32	70
19	19	9	28	76	16	21	11	32	70
19	17	11	28	76	16	16	19	35	69
21	15	5	20	76	16	32	22	54	68
21	10	11	21	76					

[a] The prepupal period (period from formation of cocoon to appearance of pupa) and pupal period occurring in cocoons formed Aug. 14, et. seq., were determined by opening the cocoons. Prior to Aug. 14 the same notations were made by observations through the glass of vials containing cocoons built against the glass.

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STUDIES ON CONFORMATION IN RELATION TO MILK PRODUCING CAPACITY IN CATTLE[1]

IV. THE SIZE OF THE COW IN RELATION TO THE SIZE OF HER MILK PRODUCTION[2]

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Woll (*9, 10*)[4], Peters (*8*), Grady (*4*), Nevens (*7*) reanalyzing Woll's data, and the work of several European workers have shown that as the weight of the cow increases, her yield of milk and milk solids increases. This fact holds for dairy cattle in the pure breeds and also in the dairy grades. The work of these writers further shows that the economy of production within any one breed is greater for the large than for the small cow, because of the fact that the small cow needs proportionately more feed to maintain her body weight. This difference in the requirements of the larger and the smaller cows has been interpreted as due to the difference in the surface areas of their bodies. Thus, while the surface area of the larger cows is greater than that of the smaller cows, proportionally to their weight the surface area of the bigger animals is much less. As heat radiates from the body surface, the food units necessary for the larger cows would be proportionally less per given unit of body area. As the cow's capacity to handle quantities of food increases with her size, the amount of food left for milk production after maintaining the body weight is proportionally greater for the large than for the small cow.

Further research may show these assumptions to be not entirely correct. Harris and Benedict (*5*) have shown that the surface-area formula for basal metabolism in men and women is not so accurate as a height, weight, and age formula. There may well be other factors of deeper physiological significance which really govern the basal metabolism of the animal, factors which in our crudeness we are interpreting as causative when they are only correlated with the basic factor. Be that as the future may show, it is of interest to extend the work of these investigators to other measures of size in cattle.

The Holstein-Friesian Cattle Club (*6*) in the early history of their Advanced Registry took a rather extensive series of measurements on their cattle. There are 385 of these records, the cows ranging in age from 1 year and 6 months to 10 years and 6 months. All records for measurement were taken within the year in which the 7-day lactation record was made. These records had measurements on height at shoulders, height at hips, body length, rump length, body width, thurl width, and body girth. The weight, either actual or estimated, was given in 339 of them. All records contained the 7-day milk yields and butter-fat percentages. The age also was recorded when the test was made. The method followed in making the measurements herein recorded conform to the following rules:

> The animal must be brought to stand on a level place and in a natural position, the feet squarely under the body, the head at a medium height, and the neck straight. The two items of height are taken perpendicularly from the ground to the top of the animal, the one immediately over the knee and center of the shoulder, and the other over the hook bone to center of the back; the length of body is taken from the extreme front of the shoulder point to the extreme rear and highest point of the rump, diagonally in a straight line; the length of the rump, from the extreme front side of the hook bone to the extreme of the rump as described above; the width of the hips, from the outside of one hook bone to the outside of the other in a straight line; the width of the thurl from the outside of one thurl bone to the outside of the other, also in a straight line; the girth by a tape closely fitting the smallest circumference of the chest; in the latter measurement the tape must be drawn so closely that a slight movement of it will move the skin of the animal. If the head is lowered from the natural position, this measure-

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[2] This paper is one of a series of investigations in animal husbandry, the continued prosecution of which has been made possible by a grant to the author from the Rockefeller Institute for Medical Research.

The data for this paper are taken from the Holstein-Friesian Advance Registry records. They include records for the 7-day milk yield, age of the cow at time of test, height at shoulders and hips, body and rump length, body and thurl width, girth, and weight. All measurements are taken according to the rules prescribed by the association and by men designated as inspectors.

[3] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 168.

[4] Reference is made by number (italic) to "Literature cited," p. 869.

ment will invariably be erroneous. And if the neck is turned to the right or left, the measurement of length of body will also be erroneous. Then follows the ascertaining of the weight, which must invariably be obtained by reliable scales; the date of service is then ascertained from the owner and the condition described. The date of the examination should also be recorded in some convenient place in the memorandum book.

The necessary analyses of these data show the averages and coefficients of variation seen in Table I.

The averages for age, butterfat percentage, and milk yield show that this group of cows is younger, has a higher butter-fat test, and is less productive of milk than are the cows of the whole Advanced Registry.

The series of means of the different parts of the body gives an idea of what might be called the "average" Holstein-Friesian cow. This average cow may or may not be a desirable specimen. It would be interesting to see a reconstruction based on these measurements. It might be found that the average is disproportionate for some of the parts, as was the case for the typical American soldier.

The weights of the cows are either estimated or actual. There are 161 records of actual weight and 178 of estimated weight. The actual weights have a mean of 1,078, a standard deviation of 162, and a coefficient of variation of 15.0. The distributions are consequently closely similar to each other.

The measurements of this average cow are, on the whole, quite a little less than those of the present Holstein-Friesian ideal. Thus in shoulder height the ideal is nearly 2 inches taller, in body length nearly 10 inches longer, in rump length 2½ inches longer, in body girth about 15 inches greater, in hip width 2 inches greater, in thurl width 2½ inches larger, and in weight about 200 pounds greater. Only in hip height do the average and ideal correspond.

The variation of the different parts of the cow is of considerable significance. The characters used in score-card judging are essentially divisible into two classes, physiological and morphological. The writer has studied the records of over 1,600 score cards taken by more than 200 judges on Jersey Registry of Merit cattle (*1*, *2*). The judges who made these scores have undoubtedly taken cognizance of the increases in size of the parts scored as age advances, and compensated for such increase in recording their judgment on the cows. The amount of variation would consequently be expected to be less than it would have been had age been left out of consideration. The data on the Holstein-Friesian cattle are actual measurements. In general, the comparison of these actual measurements with the scores shows that the scores of the judges on conformation are actually more variable than are the parts of the cows from which their scores are taken. In other words, the judgment by eye is, on the whole, less reliable than estimates based on the yardstick.

TABLE I.—*Means, standard deviations, and coefficients of variation for the physical measurements of Holstein-Friesian cows*

Characters	Mean	Standard deviation	Coefficient of variation
Age	3.90±0.08	2.22±0.05	56.9±1.8
Milk yield	338 ±2	72.6 ±1.8	21.5± .6
Shoulder height	52.8 ± .1	1.94± .05	3.7± .1
Hip height	53.8 ± .1	1.91± .05	3.6± .1
Body length	62.0 ± .1	3.73± .09	6.0± .2
Rump length	20.4 ± .1	1.44± .04	7.0± .2
Body width	21.3 ± .1	1.72± .04	8.0± .2
Thurl width	19.0 ± .1	2.63± .06	13.8± .3
Body girth	73.2 ± .2	4.59± .11	6.4± .2
Weight	1088 ±6	164.0 ±4.2	15.1± .4

The comparable results found on other forms in conjunction with those found for these Holstein-Friesian cattle are worthy of record. The weight of these Holstein-Friesian cows has practically the same variation as the weight of men or of the domestic fowl. Shoulder or hip height in cattle is slightly less variable than length of the forearm or femur in man. Rump length, body girth, and body length are slightly more variable than stature in man.

Table II reveals several facts of no little importance in the selection of dairy cattle. Every one of the variables has a rather large positive relation to the quantity of milk secreted by the mammary gland. As shown elsewhere

(*3*) a fairly large element in this correlation is the fact that the age of the cow materially influences her milk production and at the same time and in nearly like measure causes a growth in the size of the body parts. On the other hand, if one has little or no knowledge of the age of the cow and selects from a group the largest, for example, in body length or girth, one is quite sure of choosing the cow which is producing the most milk.

These facts are brought out graphically in Figure 1, which shows the average milk yield for the different classes of cows as indicated by body measurements. The size of the cows is judged by the size of the eight different measurements on the cow's body shown in Table II. The heavy lines of the figure represent the majority of the data. All the body measurements appear to be related to milk yield in a linear manner. The relation between milk yield and age appears to be linear up to about five years, when the average milk yield remains constant for the other ages. Two peculiar results are noticeable.

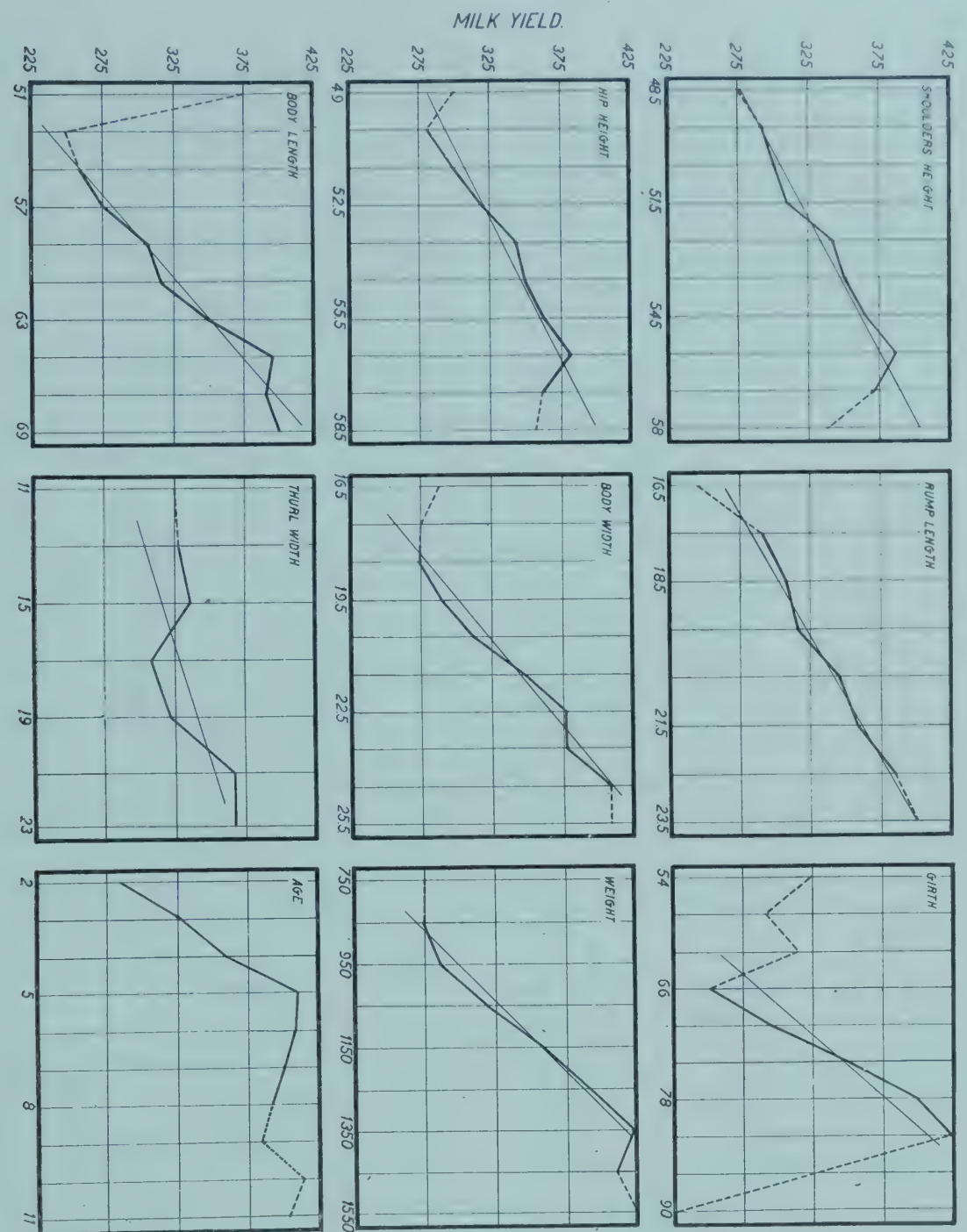

FIG. 1.—Relation of body measurements and age to the milk yield of the cow. The measurements are in inches, the weights in pounds, and the ages in years. Those points of doubtful significance, depending on less than 10 individuals, are shown in dotted lines.

TABLE II.—*Correlation of age and body measurements with the milk yield of the cow*

Characters	Correlation coefficient
Age	0.592±0.022
Shoulder height	.380± .029
Hip height	.344± .030
Body length	.590± .022
Rump length	.391± .029
Body width	.520± .025
Thurl width	.214± .033
Body girth	.476± .027
Weight	.652±[a].021

[a] The correlation for the 161 actual weights for milk yield with body weight is 0.623.

The graph showing the relation of milk yield and body length has as its first observation a cow of short body length but of relatively high milk yield. As this average is based on only one individual it can scarcely be significant except in showing the amount of variation which can occasionally be found when considering individual cases. The graph for girth shows similar irregularity. The drop from the high point of milk yield with a girth of 82 inches to the lowest point in milk yield with a girth of 90 inches is based on one individual. It seems highly probable that there was an error in this measurement. However, as this is not known, the data are used as given. The irregularity for the first part of this curve likewise is due to very limited data.

The prediction equations derived from the correlation coefficients and the means and standard deviations of the data are shown by the straight lines. It is evident that these straight lines follow the major portion of the data very well for all cases except those for girth and age. The equation for girth is, as noted, very materially influenced by the irregularities of the first three observations and also the last. If these observations should be discarded it would be found that the resulting equation fitted the data for 66 to 82 inches of girth almost exactly. In the case of age it has not seemed desirable to calculate the linear line, as it is well known that this line does not fit the relation between age and milk yield. It has therefore been omitted from the original chart. It might be said, however, that in attempting to obtain the relation between the body measurements for a constant age this linear line might be used, as it would correct for most of the variability caused by age. This method of correction is given elsewhere.

Judging from these graphs it seems entirely reasonable to use straight lines to represent the increase of milk yield for the increase in body size. The linear equations for these data as noted above have therefore been calculated. These equations are given as follows:

(1) 7-day milk yield=14.2 shoulder height−412.9
(2) 7-day milk yield=13.1 hip height−366.5.
(3) 7-day milk yield=11.5 body length−374.1.
(4) 7-day milk yield=19.7 rump length−63.5.
(5) 7-day milk yield=21.9 body width−128.3.
(6) 7-day milk yield=225.8+5.9 thurl width.
(7) 7-day milk yield=7.4 girth−201.5.
(8) 7-day milk yield=23.8+0.289 weight.

These equations are used for the straight lines of Figure 1. We may note certain of the significant points connected with them. Thus for each inch increase in height at shoulders there is an increase in 7-day milk yield of more than 14 pounds. For each inch of increased height at hips there results an increase of milk yield of 13 pounds For an increase of body length of 1 inch there results an increase of milk yield of 11.5 pounds on the average. An increase of 1 inch of rump length represents an increase of milk yield of 19.7 pounds and an increase of body width at hips of 1 inch increases the average milk yield of the cow 21.9 pounds. An increase in width at the thurl represents a lower increase in milk yield, for 1 inch increase in thurl width is only equivalent to 5.9 pounds increase in milk yield. An increase of 1 inch in girth represents an increase of 7.4 pounds of milk on the average for such cows. An increase of 1 pound of weight represents an increase of milk yield of nearly 0.3 pound of milk.

These results can not, of course, be compared directly, as the range of variation for the various body measurements is markedly different. If what might be reasonably considered the end points of these different measurements as indicated by significant data are taken, it is found that the body length, body width, girth, and weight are the items which caused the most marked increases in the 7-day milk yields of the cows, as the measurements are varied from the lowest to the highest in the breed. Closely following these are rump length, height at shoulders and hips. The thurl width is the least important measurement.

It is of interest to compare the amount of variation in the milk yields controlled by these different body measurements. It will be remembered that the reduction in standard deviation due to the making constant of

any given variable is equal to the original standard deviation minus the original standard deviation times the square root of 1 minus r square ($SD—SD\sqrt{1—r^2}$). When the height of shoulders is made constant, the variability in milk yield of Holstein-Friesian cattle is reduced 5.4 pounds. When the height at hips is made constant, the variability is reduced 4.4 pounds. When the body length is made constant the variability is reduced 14 pounds. A constant rump length reduces the variation in milk yield 5.8 pounds. A constant body width reduces the variation in milk yield 10.6 pounds. A constant thurl width reduces the variation 1.7 pounds. By making the girth constant the reduction is 8.7 pounds in milk yield. A constant weight reduces the variation in milk yield 17.5 pounds.

From these data it is noted that body length, body width, girth, and weight are the most important items in relation to milk yield and its variation. Weight is the most important single element, but is closely followed by the body length and body width. Were just the significant measurements for girth to be taken it would be found that this measurement is nearly if not as important as that of body length. The figures given in the above paragraph may be inverted and the results expressed in this way. For cows of a constant weight the 7-day Advanced Registry records of Holstein-Friesian cattle would vary only to 77 per cent of the extent to which they now do. If the cows were of a constant body length the variation in milk yield would be only 80 per cent of that now found. In the same way we could convert the figures for the other measurements on the size of the cow.

SUMMARY

The results show that, for these cows at average age of 3.9 years and a milk yield of 338 pounds for the 7-day period, the average measurements are shoulder height, 52.8 inches; hip height, 53.8 inches; body length, 62.0 inches; rump length, 20.4 inches; body width, 21.3 inches; thurl width 19 inches; body girth, 73.2 inches; and weight, 1,088 pounds. The variations of these measurements are comparable to those taken on the body parts of other animals. The coefficients of variation range from 3.7 to 15.1. The most variable part is weight. The least variable is height at hips.

All of these body measurements are related to milk yield, so that an increase in any one of them results in an increase of 7-day milk yield. The relation of all measurements to milk yield is linear for these data. The most important element predicting milk yield is weight. Body length, body width, or girth, closely follow weight in the accuracy with which they predict milk yield.

Increases of one inch produce an average increase in milk as follows: Shoulder height, 14 pounds; hip height, 13 pounds; body length, 11.5 pounds; rump length, 19.7 pounds; body width, 21.9 pounds; thurl width, 5.9 pounds, girth, 7.4 pounds. An average of 100 pounds in weight results in an average increase of 29 pounds of milk.

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NECROSIS, HYPERPLASIA, AND ADHESIONS IN MOSAIC TOMATO FRUITS[1]

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INTRODUCTION

The extremely severe streak or winter blight type of mosaic occurred in a greenhouse crop of Bonny Best tomatoes at La Fayette, Ind., during November and December, 1923, and an opportunity was afforded to make a preliminary study of the structural abnormalities exhibited by many of the diseased fruits. The disease was characterized by the destructive necrotic streaking and spotting of leaves, stems, and fruits described in a previous account (*12*, *p. 8*).[3] Every one of the more than 800 plants in the house also evinced on the young leaflets the typical dark green puffy areas on a lighter background, a reliable diagnostic feature of the mosaic type of disease. The mosaic nature of the disease was further verified by successful inoculation of healthy tomato seedlings with the juice of a young fruit by J. B. Kendrick in another house and by numerous cultural tests which proved the internal fruit lesions to be free from bacteria.

Hundreds of fruits of all sizes and ages showing a variety of responses to the disease were available for study, and unstained free-hand sections of fresh material, mostly from very young green fruits, revealed most peculiar abnormalities in the tissues. The sections were mounted in water and preserved by adding lacto-phenol (equal parts of phenol, lactic acid, glycerin, and water). Material was also embedded in paraffin, sectioned on the microtome, and stained with gentian violet, safranin and licht green, Haidenhain's iron-alum haematoxylin, and Ziehl's carbol fuchsin.

NORMAL ANATOMY OF THE FRUIT

An acquaintance with certain phases of the normal anatomy and development of the fruit is a necessary preliminary to a consideration of the abnormal. The mature tomato with its several-celled ovary and fleshy walls and its bulky axile placentae bearing the seeds embedded in a pulpy matrix of thin-walled parenchymatous tissue is familiar to all. A very young ovary about 4 mm. in diameter as shown in Figure 1, A, differs mainly in that the ovules more or less completely fill the locular cavity and are not embedded in a cellular matrix. During the early stages of enlargement of the fruit the placental tissue grows out between the ovules (fig. 1, B) to form the gelatinous matrix of thin-walled parenchyma which separates and finally engulfs the ovules and completely fills the locular cavity (fig. 1, C and D) by the time the fruit is about 10 mm. in diameter.

The placental matrix touches the inner surface of the fruit wall or pericarp and the surfaces of the radial locular partitions, but remains free from these surfaces as well as from the epidermis of each ovule (fig. 1, B, C, and D). The young fruit enlarges very rapidly under greenhouse conditions and hence is composed of very active meristematic tissue. Figure 1 shows that growth must progress with extreme rapidity in the placental matrix in the short period during which the ovary enlarges to 1 cm. in diameter. Later the epidermal cells of the seed coat elongate enormously to form a palisade layer, which touches but remains free from the placental matrix.

GROSS ABNORMALITIES IN MOSAIC FRUITS

One of the most conspicuous symptoms on mosaic fruits is the eruption of brownish, translucent, rather flat-topped blisters of various shapes and sizes as illustrated in Plate 1, B, and in a previous paper (*12*, *p. 8*). These may later be bordered by a shallow peripheral fissure. Large lesions of this type may eventually contain numerous cuticular fissures and form

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[3] Reference is made by number (italic) to "Literature cited," p. 888.

roughened buckskin areas on the fruit as described and illustrated by McKay (*18, p. 181*). Mosaic fruits are also characterized by more deep-seated lesions, producing an irregular pattern of hard, sunken, brown or black pockmarks, as shown in Plate 2, E and F. Ripening fruits often show rather extensive sunken granular areas which fail to color properly and under which the seed pulp or placental matrix tends to remain greenish.

Ovaries and very young fruits may show brown epidermal or deep-seated

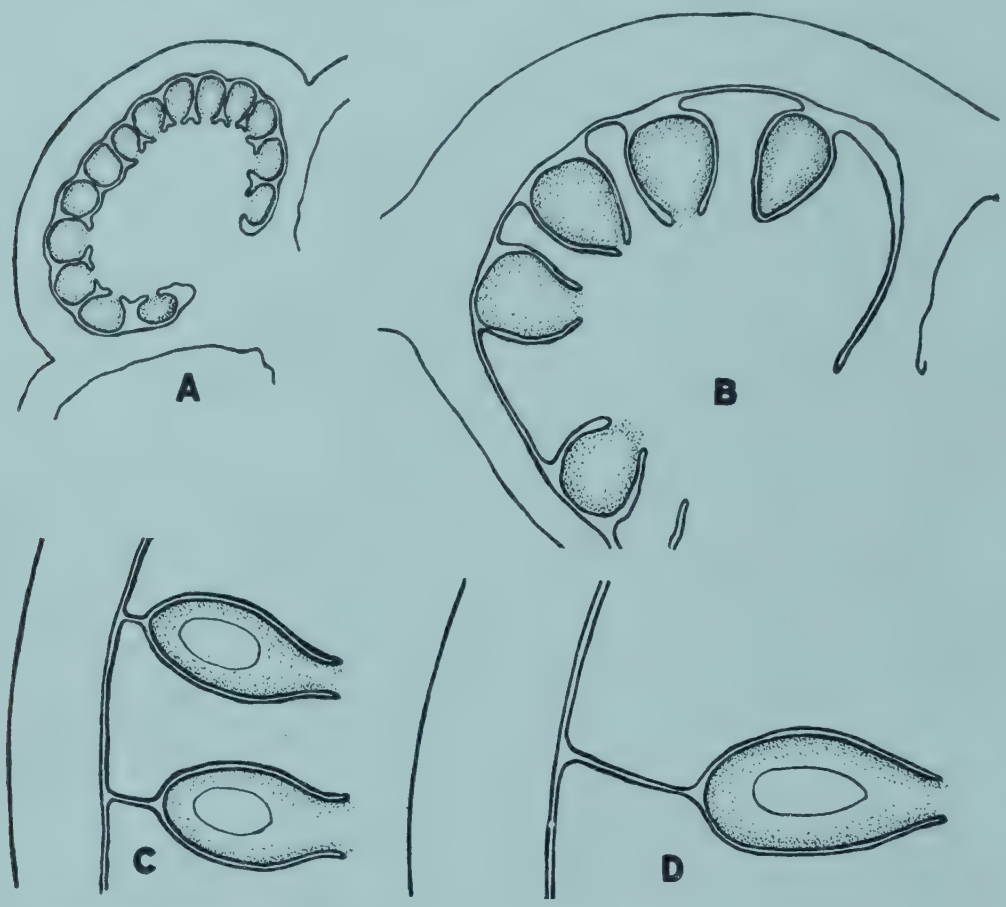


FIG. 1.—Origin and development of the placental matrix in which the seeds in a tomato fruit are embedded. Camera lucida diagrams drawn to the same scale (×15) to show how the placental tissue grows out between and around the ovules, finally engulfing them completely, during a very short period early in the growth of the fruit

A.—Locule of an ovary 3 mm. by 4 mm. in diameter showing ovules seated upon the surface of the axile placenta and completely filling the locular cavity

B.—Locule of a small fruit 5 mm. by 6 mm. in diameter showing how the placental tissue has grown out between the ovules into contact with the pericarp, thus surrounding and separating the ovules and filling the locular cavity as the latter enlarges. The ovules are receding from contact with the pericarp

C.—Portion of locule in fruit 7 mm. in diameter showing how the placental tissue has completely engulfed the seeds as the latter recede from actual contact with the pericarp. This placental tissue develops very rapidly and completely fills the enlarging locular cavity

D.—Portion of locule in fruit 10 mm. in diameter showing distance to which pericarp has grown from the seed, the intervening space being filled by a cellular matrix resulting from the outgrowth of the placenta. It is in this placental matrix that the abnormalities predominate in mosaic fruits. The growth of pericarp and seed may also be noted

EXPLANATORY LEGEND FOR PLATE 1

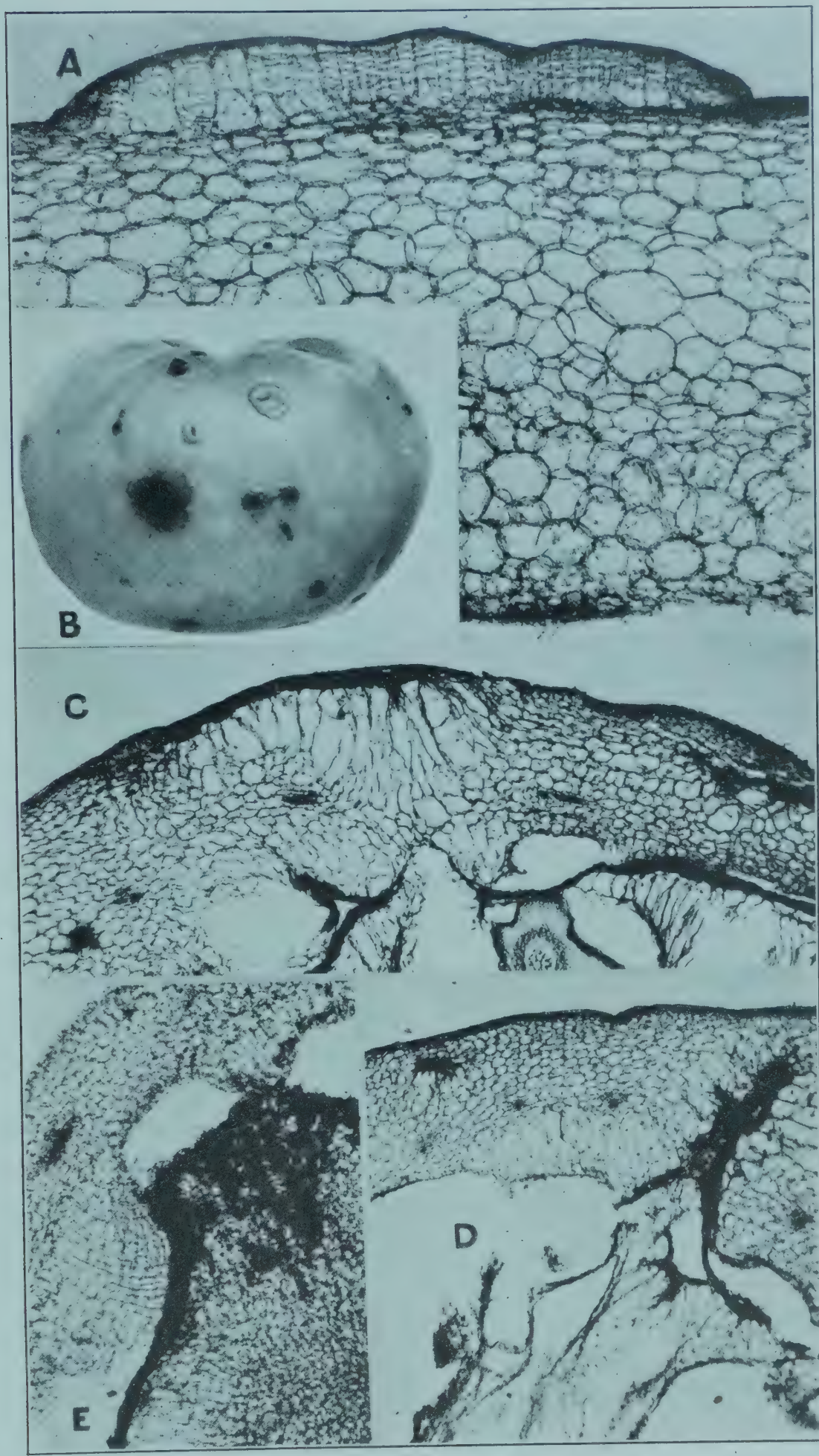
A.—Section of pericarp through a surface blister such as is illustrated in B, showing the hyperplastic character of the blister tissue. The subepidermal cells have elongated and divided, pushing up the epidermis. If this tissue collapses the blister sinks and the epidermis usually cracks where it is sharply curved upward at the edge of the blister, thus forming the marginal fissure noted about the older lesions. Photomicrograph × 33. Unstained

B.—Surface blisters on a mosaic tomato

C.—Section of a pericarp lesion resulting from necrosis and collapse of an area of subepidermal cells and hypertrophy of the cells immediately beneath the necrotic area. The necrotic surface of the underlying placental matrix, to which an ovule is adhering, is in turn adhering to the inner surface of the pericarp. Photomicrograph × 33. Stained with Haidenhain's iron-alum haematoxylin. Fruit 12 mm. in diameter

D.—Section of pericarp showing at the right a transverse necrotic plane accompanied by elongation of the near-by cells. At the left is a small necrotic spot in the pericarp. Photomicrograph × 33. Stained with carbol fuchsin. Fruit 12 mm. in diameter

E.—Intumescences from a radial locule partition extending into an abnormal cavity which has resulted from the collapse of the necrotic placental tissue. Photomicrograph × 46. Unstained. Fruit 7 mm. in diameter



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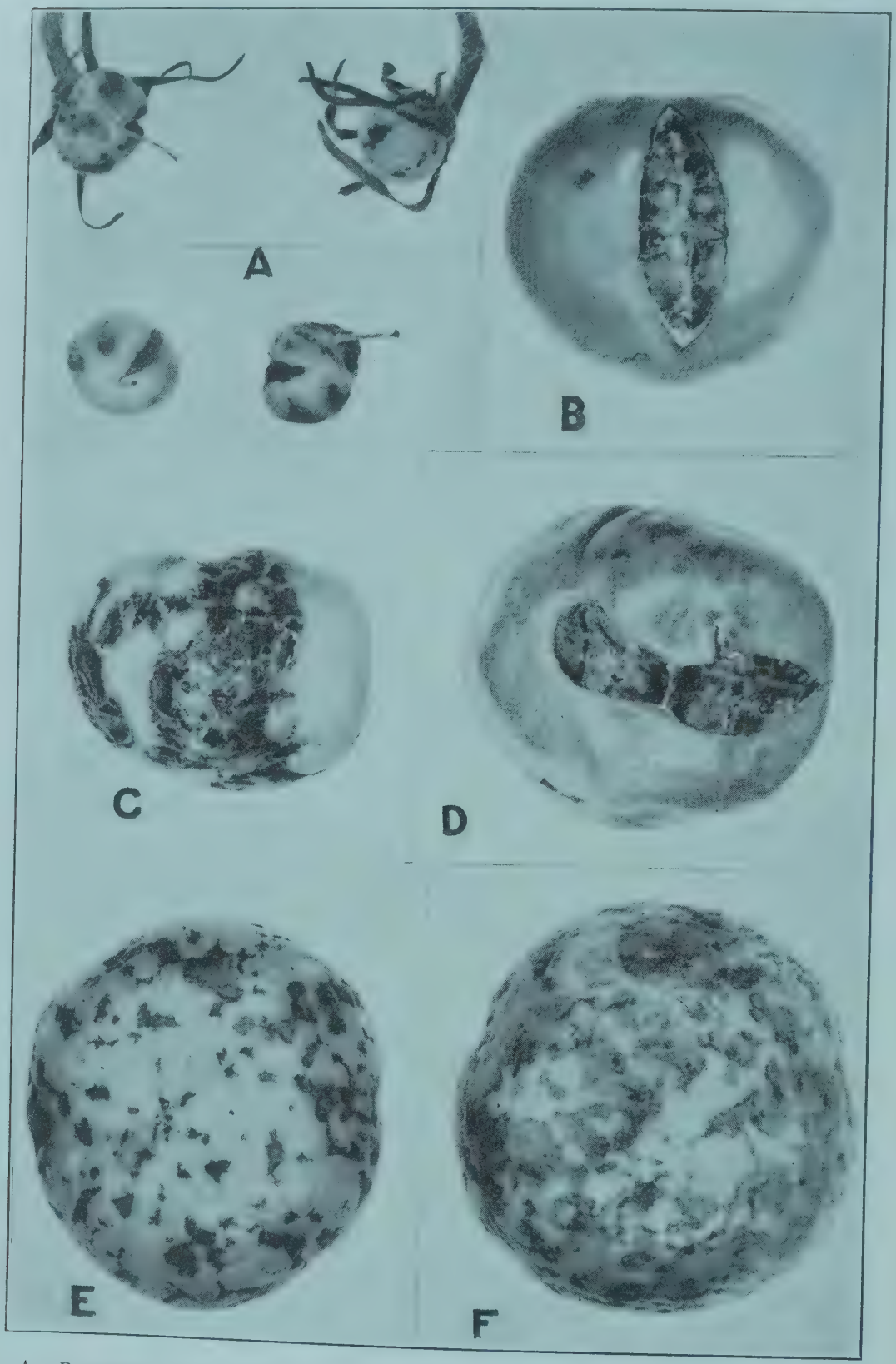


A.—Four very young tomato fruits showing necrotic lesions. Two show rupture of the pericarp and would probably develop into such fruits as those shown in Plate 3, C

B.—Longitudinal rupture of the pericarp, as a result of an earlier necrotic lesion, exposing a considerable area of the seed pulp or placental matrix

C.—Scabby lesions stunting growth and causing deformity and pericarp rupture in young fruit

D.—Transverse rupture of the pericarp as a result of an earlier necrotic lesion

E.—Scattered, slightly sunken surface lesions or pockmarks

F.—Coalescence of lesions producing a brownish pattern with a hard, sunken, granular surface. Such fruits fail to color properly

markings without the blister effect (pl. 2, A) and in some cases the entire surface of the fruit is involved, a condition associated with early abscission or invasion by rot-producing organisms. The discoloration may sometimes be internal and deep seated so as to show through the outer pericarp layers as an evident darkening of the underlying tissues (pl. 3, B).

The surface lesions may become hard, dark brown scabs which inhibit the symmetrical growth of the fruit and as a result produce marked malformation, as is shown in Plate 2, C. Often the brown necrotic lesions involve the pericarp so deeply that, after the affected tissues collapse, the weakened areas are ruptured by the growth pressure of the interior tissues, and large irregular openings are produced in the pericarp exposing the seeds and placental matrix to the drying effects of the air (pl. 2, B and D). In fruits thus affected when very young, as shown in Plate 2, A, mere shreds of the pericarp may remain as is shown in Plate 3, C, or deep cracks may result as shown in Plate 3, D.

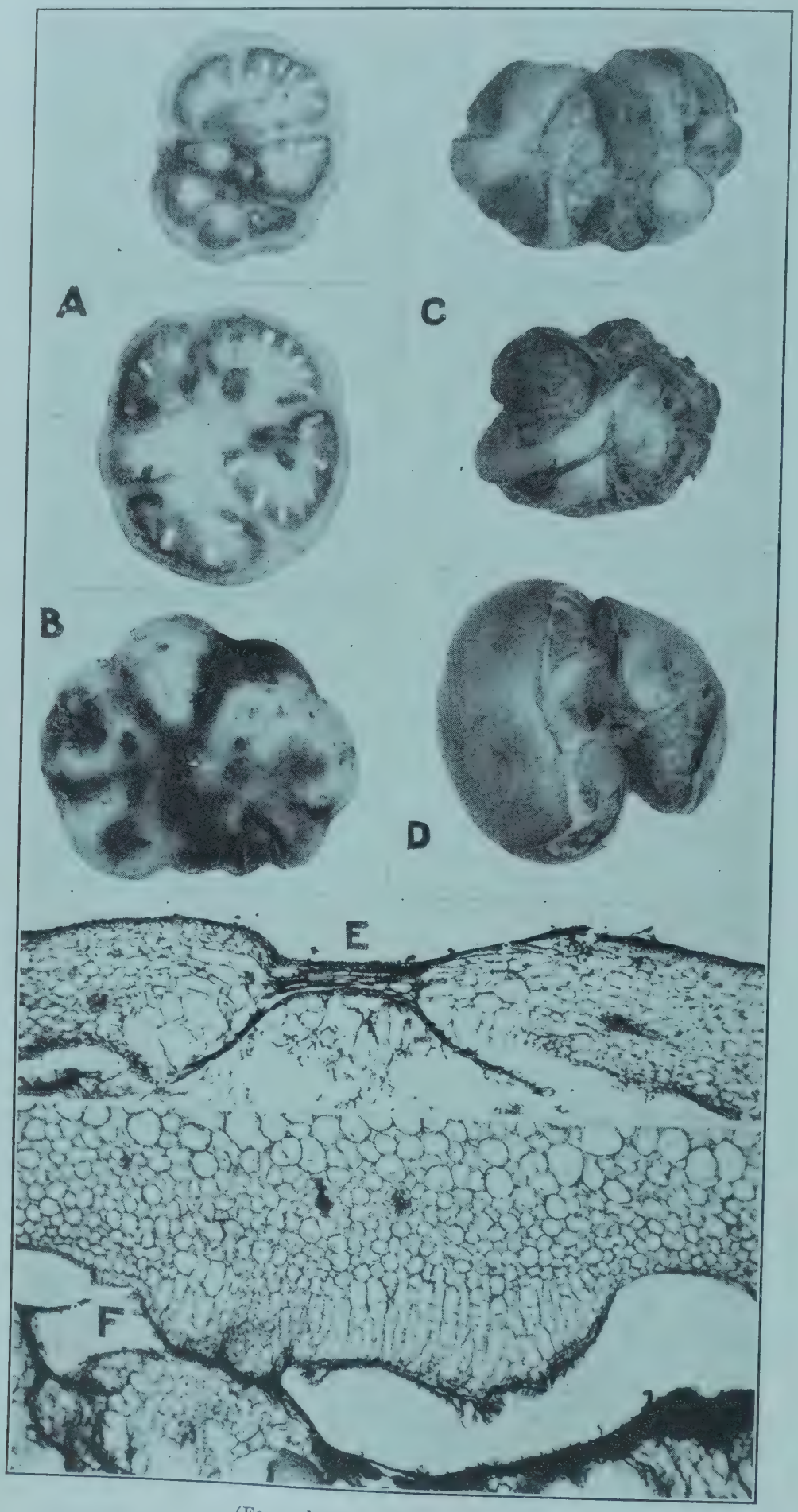
When the fruit is cut across it is found that brown necrotic regions usually occur throughout the interior tissues if there are any external lesions on the specimen (pl. 3, A) and in cases of extensive surface discoloration all or considerable portions of the interior may also be similarly discolored. Ordinarily, however, the internal necrosis occurs in the form of scattered strips, pockets, or thin layers, not continuous or connected with each other nor, as a rule, sufficiently extensive to completely inhibit the enlargement of the fruit, although its normal development may be seriously interfered with. The necrotic regions occur most abundantly in the placental matrix that fills the locules, and the most prevalent type consists of rather extensive necrotic planes parallel to and in rather close proximity to the locule walls. These necrotic regions are usually but not always surrounded or bounded by zones of rather firm, glassy or translucent tissue. In young ovaries many of the ovules are entirely necrotic and atrophied. This occurs usually in only part of the locules and often involves only part of the ovules in the locule. Many mature seeds show brown spots.

Examination of razor sections of the young fruits with a hand lens reveals very clearly the necrotic regions and the translucent zones associated with them and also shows rather extensive cavities associated with the necrosis of the distal portions of the placental matrix. These cavities may be entirely surrounded by a brown necrotic lining as is the small one in Plate 4, A, or may occur between the necrotic placental surface and the locule wall as in Plate 1, E. Furthermore many of the seeds may be displaced or abnormally located and oriented in the placenta, and the normal arrangement of the placental matrix as shown in Figure 1 is very generally interfered with to a greater or less extent. It is plainly evident also that necrosis often occurs within the seeds, that some ovules are affected more than others, and that various degrees of retardation in the development of the seeds and the placental matrix occur in the same fruit and even in the same locule. For example, certain locules in fruits 5 mm. and 7 mm. in diameter showed necrotic atrophy of the ovules and complete suppression of any development of the placental matrix beyond the stage shown in Figure 1, A.

NECROSIS, HYPERTROPHY, AND HYPERPLASIA

Microscopic examination of unstained sections shows that the surface blisters are produced by a compact muriform cushion of meristematic cells which have pushed up the epidermis (pl. 1, A). Epidermal pustules or intumescences of all sizes are found, the smaller ones being papillate rather than flat-topped. In the incipient stage of a blister, necrosis of the epidermal cells is visible and the hyperplasia is seen to originate in the first and second subepidermal cell layers (pl. 5, A). The translucent quality of the blisters is very evidently attributable to the lack of intercellular spaces in this hyperplastic tissue (pl. 5, B).

Zones of brown necrotic cells of varying shape, extent, and orientation are of common occurrence at varying depths in the pericarp, producing, as a result of collapse of the necrotic cells (pl. 6, B and C), dark sunken pockmarks on the exterior of the fruit. Such zones often involve the entire thickness of the pericarp and, as a result of early atrophy of the tissues (pl. 6, A) or collapse of the necrotic cells (pl. 3, E; pl. 6, C), produce weakened places in the pericarp which are easily ruptured by growth pressure. This results in such fruits as are shown in Plate 2, B and D, and Plate 3, C and D. Similar necrotic regions occur scattered promiscuously through the locule walls and the placental tissue,



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and atrophied or necrotic weakened places occur commonly in the locule walls. Necrotic regions are found within the endosperm of the ovule and occasionally in the embryo, and complete necrosis and atrophy of certain ovules is of common occurrence. The brown spots on the seeds previously mentioned are due to necrotic areas in the outer part of the endosperm just under the seed coat. The occurrence of these symptoms within the seed is of peculiar interest in view of the fact that the disease apparently is not seed-transmitted.

Necrotic tissue is most abundant in the peripheral regions of the placental matrix (pl. 4, A; pl. 6, C). The more extensive necrotic areas seem to originate very frequently in epithelial tissues such as the lining of the locule and the surfaces of the placental matrix. In almost all cases except within the endosperm of the seed the necrotic cells soon collapse (pl. 6, D) and the necrotic tissue frequently surrounds or borders upon cavities of varying extent resulting from the collapse and shrinkage of the cells (pl. 1, E; pl. 3, F).

The collapsed necrotic regions are practically always accompanied and bordered or more or less surrounded by zones of closely packed hypertrophied cells elongated toward the necrotic region or by zones of hyperplastic tissue composed of more or less parallel columns of somewhat rectangular, closely packed, meristematic cells (pl. 1, C and D; pl. 6, C and D). This tissue apparently arises from the living parenchyma cells bordering on the necrotic region and is characterized by reduced intercellular space, a condition which accounts in part for the translucent appearance of these zones (pl. 4, A). These cells by radial elongation and frequently by transverse division produce the columnar tissue which grows and pushes in toward the necrotic region (pl. 7, B). Numerous instances of marked spherical hypertrophy of individual cells and radial hypertrophy of groups of adjacent cells in the epithelium lining the locular cavity have been noted opposite necrotic ovules in young ovaries. The latter condition is interpreted as the incipient stage of hyperplastic growth.

When the necrosis occurs in the interior tissues of the pericarp, locule wall, or placenta, the response of the surrounding tissue is largely hypertrophy alone, the cells enlarging and elongating in toward the necrotic region as the latter collapses and shrinks (pl. 1, C and D; pl. 6, C and D). Elongation of endosperm cells also occurs. On the other hand, when the necrosis occurs in the epithelial tissues, such as the epidermis, the epithelium lining the locules, and the epithelium of the placental matrix, the response of the underlying and adjacent cells, particularly those of the pericarp and locule wall, is elongation followed by transverse division with the resultant production of intumescences (pl. 1, E; pl. 3, F; pls. 4, 5, 7, and 8).

At the bases of the hyperplastic growths, or intumescences, hypertrophied cells are very conspicuous, while the cells in the distal portions or advancing faces of these hyperplastic growths, the part nearest the necrotic tissue, are much smaller than normal cells (pl. 5, B; pl. 7, A and B; pl. 8, A). In the larger growths, or intumescences, the parallel nature of the cell columns may become deranged by the pressure from basal groups showing renewed or more rapid growth, possibly in response to secondary necrotic areas within the intumescence (pl. 7, B). In the basal region of hyperplastic zones originating in the placental matrix, the cell contents are dense and brownish (pl. 4, A and B).

The most extensive development of these hyperplastic zones is on the inner surface of the pericarp and on the locule walls, particularly at the outer angles of the locule, in response, it would appear, to peripheral necrosis of the adjacent placental matrix or to necrotic

EXPLANATORY LEGEND FOR PLATE 3

A.—Two young fruits cut across to show internal necrotic regions and translucent zones about the lining of the locules and in the placentae and pericarp

B.—Badly affected young fruit showing rupture of pericarp and deep-seated internal brown discoloration along the sutures. Such fruits contain an abundance of internal necrosis and hyperplasia

C.—Two ruptured and deformed fruits with mere remnants of a pericarp as a result of necrotic or atrophic lesions in the young ovary wall, such as are shown in Plate 2, A

D.—Deep cracking of a fruit that was affected with necrotic lesions when very young

E.—Section of a pericarp lesion showing the collapse of a subepidermal necrotic region involving the entire thickness of the pericarp. At either side hypertrophied cells may be seen, and the placental matrix bounded by a necrotic plane has pushed up under the pericarp lesion. Such a lesion would be ruptured by the subsequent growth of the fruit. Photomicrograph × 33. Stained with carbol fuchsin. Fruit 12 mm. in diameter

F.—Section of an intumescence on a radial locule wall extending in toward the necrotic surface of the placental matrix and adhering to the latter at one point. The hyperplasia originates in the subepithelial layers. Photomicrograph × 33 from longitudinal section of a fruit 12 mm. in diameter, stained with Haidenhain's iron-alum haematoxylin

(For explanatory legend see p. 879)

areas in the epithelium lining the locule. Internal intumescences of considerable size were found in the larger green fruits. Early stages in the growth of such intumescences are shown in Plate 1, E, and Plate 3, F, and larger ones are shown in Plate 7 and Plate 8, A. Plate 7, A, represents a very large intumescence, a double outgrowth which measured about 2 mm. in depth. Most of them are not much over 1 to 1.5 mm. in depth. Where these internal intumescences impinge upon the placental matrix there are thin plates of crushed brown cells, the necrotic planes mentioned above (pl. 5, C1; pls. 4, 7, and 8). Very often a reciprocal and opposite hyperplastic zone has arisen in the placental tissue meeting the invading intumescence (pl. 4, A) or pushing up against the pericarp (pl. 3, E; pl. 6, B) on the inner side of the necrotic separation plane.

The impression is gained from the examination of the sections that the intumescences from the inner wall surface actually invade and crush the placental parenchyma, exerting considerable pressure thereon (pl. 7, A; pl. 8, A), and the same condition would appear to exist at the centers of the whorls of hypertrophied cells surrounding necrotic pockets (pl. 6, D). The thin flattened condition of the necrotic separation planes may be due to the pressure of the opposing hyperplastic growths. This pressure might be one cause of the greater firmness of these regions noted when the tissues are cut. In many cases hyperplastic growths from the locule wall impinge upon ovules (pl. 5, C; pl. 7; and pl. 8, B), and in one instance noted the side of a seed was indented, apparently by the pressure of one of these growths. In a few cases, evidence of hypertrophy and hyperplasia was noted in the endosperm, and abnormal transverse walls in the palisade epidermal cells of the seed coat were noted in seeds lying near a necrotic zone in the placental tissue (pl. 8, B).

Hyperplastic tissue similar to that found in fruits has also been found in the cortical tissue of peduncles and petioles, immediately underlying necrotic surface stripes.

In the stained preparations it was found that the necrotic tissues tended to retain safranin and the living cells, licht green, gentian violet, or Ziehl's carbol fuchsin. Both retained Haidenhain's iron-alum haematoxylin.

A few cases of rounded dark green external projections or knobs on the older fruits due to hypertrophy of the interior cells of the pericarp have been found. These seem to represent a feature somewhat similar in nature to the abnormal projections on the fruit in the case of cucumber mosaic.

ADHESIONS

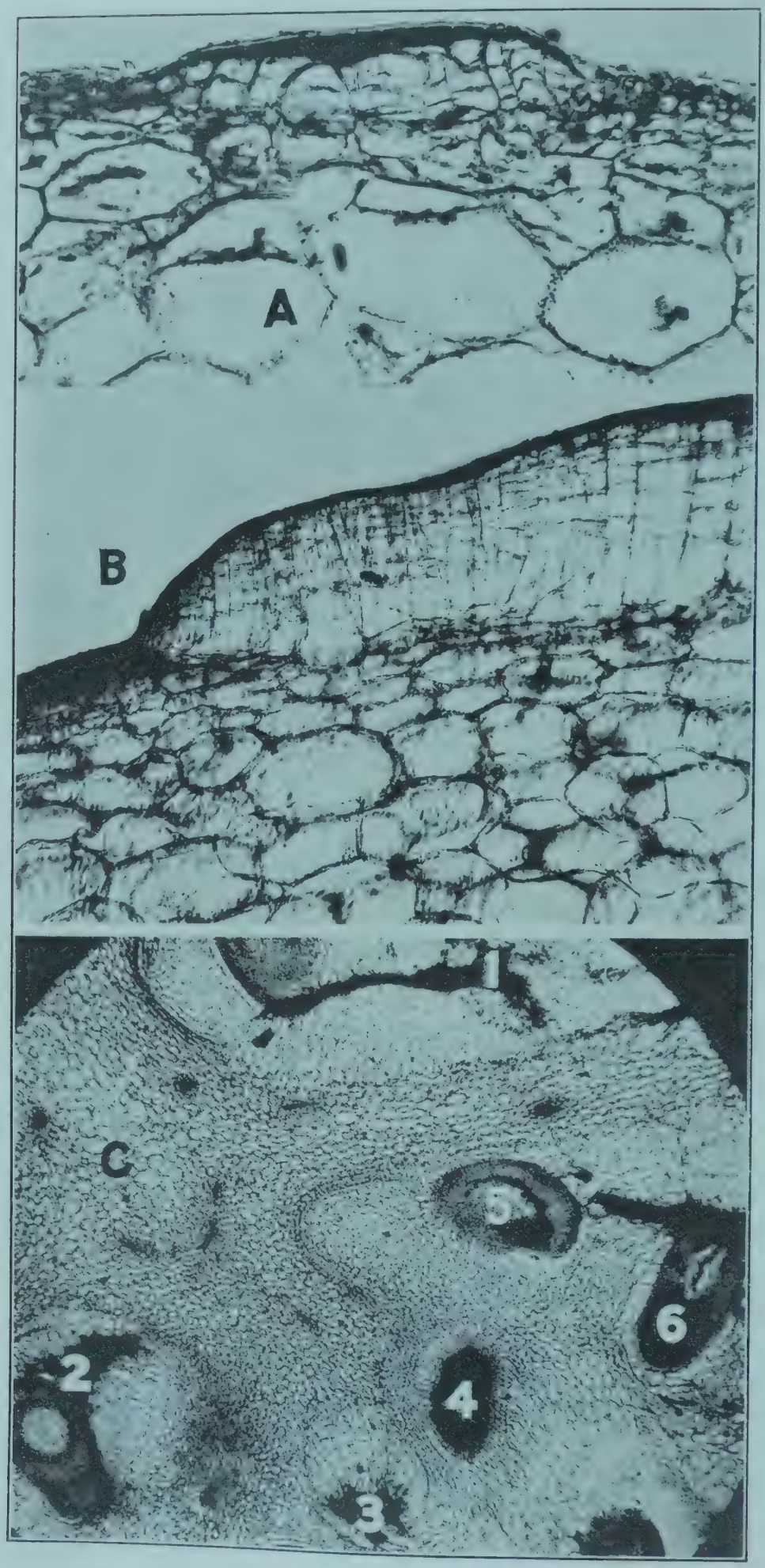
Perhaps the most interesting feature of the internal abnormalities in these mosaic tomatoes is the fact that the hyperplastic invasions result in actual and firm adhesions between the pericarp or locule wall and the adjacent placental matrix and ovules (pl. 1, C; pl. 3, F; pl. 4, A; pl. 6, B and C; and pl. 8, B) and also between the placental matrix and the epidermis of the seed coat. The intervening necrotic plane is not usually a true separation plane, but seems rather to represent a plane of adhesion. Furthermore it is not always continuous, in which case the opposing tissues appear actually to fuse or grow together by infiltration into each other (pl. 4, B).

This phenomenon of abnormal adhesions is readily demonstrated by peeling a segment of the pericarp from a locule in which case single seeds or groups of seeds surrounded by the placental matrix will pull loose from their normal funicular attachment and remain firmly adherent to the inner surface of the pericarp at many of the points where necrotic planes are located. Furthermore, these adhesions are readily recognizable in the manipulation of the razor sections. The abnormal fusions between interior surfaces add to the unnatural firmness of affected tissues, to the disarrangement of the ovules, and to the external malformations evinced by the growing fruits. In one case an unnatural groove in the fruit was attributable to an underlying adhesion at the corner of a locule.

EXPLANATORY LEGEND FOR PLATE 4

A.—Section through an adhesion in the angle of a locule in a mosaic tomato 11×17 mm. in diameter. The normal thickness of the pericarp is shown at the top. The hyperplastic growth from the placental tissue begins near the base of the ovule and far exceeds in extent the reciprocal inward growth from the pericarp and locule wall. The denser cell contents at the base of the placental hyperplasia are a common accompaniment of placental hyperplasia. The necrotic plane parallels the carpellary walls, a feature commonly noted in microscopic examination of specimens. Photomicrograph × 33. Unstained

B.—Section through an adhesion between pericarp (above) and placental matrix in which the necrotic plane is discontinuous and the hyperplastic growths from pericarp and placenta have infiltrated into each other or fused together. The dense cell contents at the base of the placental hyperplasia are similar to those pointed out in A above. Fruit 25 mm. in diameter. Photomicrograph × 33. Unstained



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DISCUSSION

From a study of these abnormal tissues the impression is gained that the hypertrophy and hyperplasia are responses to the necrosis, possibly an effort on the part of the host to repair and replace or to isolate and occlude the necrotic tissue, possibly a response to a stimulus emanating from the necrotic tissue. In the surface blisters this explanation is tenable, inasmuch as the epidermal layer is necrotic. The well-developed intumescences on the inner walls of the locules occur only opposite necrotic regions in the placental matrix or under necrotic areas of the inner epithelium of the pericarp. It may be stated as a general rule that the hypertrophy and hyperplasia occur only in close association with the necrosis.

Structurally the type of growth resembles that involved in wound tissue or cork formation. The fact that the smaller cells in the hyperplasias occur in proximity to the necrotic tissue would indicate that the latter may supply the growth stimulus. Riker (*28, p. 427*) found that the smaller cell size and the most rapid cell division in tomato stems inoculated with the crown-gall organism occurred in the cells adjacent to the intercellular spaces containing the bacteria and hence nearest to the source of stimulation. However, in the larger intumescences in the mosaic fruits there is evidence of renewed waves of growth in the basal portions quite distant from the necrotic region.

The destructive invasion of tissues by these proliferations and the abnormal tissue fusions may be interpreted as incidental consequences of the growth response to the necrotic condition. However, it may be possible that hyperplasia, as well as necrosis, is a direct effect of the mosaic virus. The flattened necrotic plates are doubtless the result of the pressure of the hyperplastic tissue.

That the epithelium lining the locules in a young tomato is readily reactive to a growth stimulus, or to a removal of growth inhibition, is shown by the ease with which Smith (*31, p. 174*) was able to produce surface proliferations by exposing this epithelium to the vapor from injections of 20 per cent solutions of ammonium carbonate, acetate, and tartrate. The greater prevalence of necrosis in the tissues of the placental matrix may possibly be associated with their extremely rapid growth.

A partial review of the literature on mosaic and related diseases shows that certain of these histological phenomena have been previously noted. With respect to the malformation and rupture of fruits, the bizarre effect of the mosaic disease on cucumber fruits has been described by Doolittle (*11, p. 13*), who also noted that the same disease caused a bursting or rupture of the pericarp of the wild cucumber fruit. Allard (*1, p. 255*) noticed malformation of the blossoms in tobacco mosaic which interfered with the normal development of the pistil and reported that mosaic reduced the number and the viability of the seeds. The malformation effect of mosaic on tomato fruit has been noted by Dickson (*10, p.* 18) and has been reported in connection with winter blight by Howitt and Stone (*14, p. 164*). Both internal and external malformation of the tomato fruit were noted by Cobb (*9, p. 412*) in a disease called rosette. The necrotic surface-spotting of tomato fruits associated with mosaic or related diseases has been described by a number of observers including Selby (*30, p. 238*), Orton and McKinney (*23, p. 242*), Howitt and Stone (*14, p. 163*), Brittlebank (*8, p. 132*), Paine and Bewley (*24, p. 187*), McKay (*18, p. 181*), Gardner and Kendrick (*12, p. 8*), and Poole (*25, p. 5*).

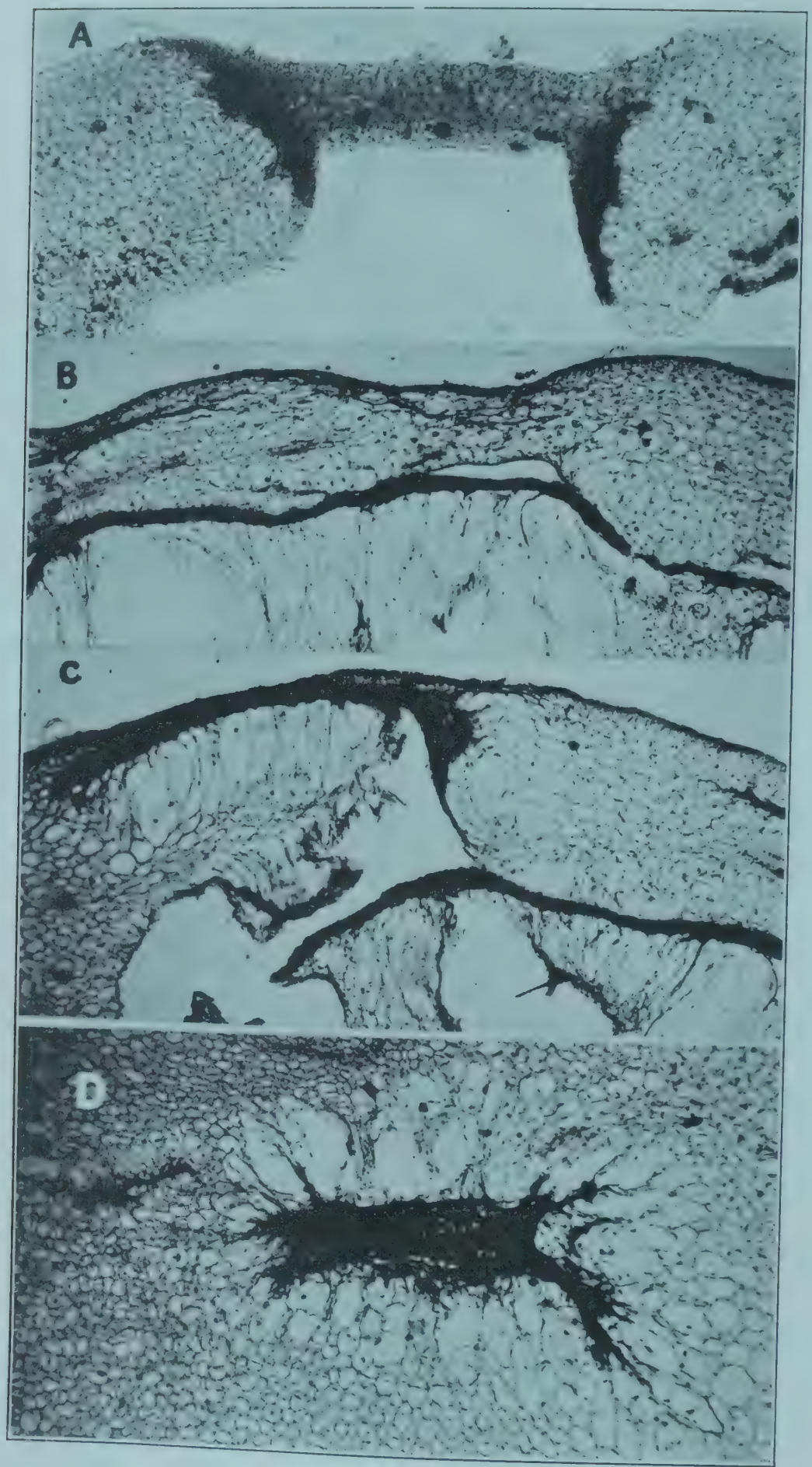
Of particular interest in connection with the surface blister lesions on tomatoes and pericarp-rupture are the observations of Atanasoff (*4, p. 8*) upon the occurrence of flat translucent blisters on very young potato tubers of the Schotsche Muis variety affected with stipple streak, in which case the blisters sink and produce hardened

EXPLANATORY LEGEND FOR PLATE 5

A.—Section of an early stage of a fruit blister showing the brown necrosis of the epidermal cells and the hyperplasia of the subepidermal cells. Photomicrograph × 126. Stained with gentian violet.

B.—Cross section of a portion of a surface blister showing radial elongation and transverse division of the subepidermal cells to form the hyperplastic tissue of the blister. The necrotic tissue is in the epidermal region of the blister. Photomicrograph × 82. Unstained

C.—Cross section near center of a fruit 1 cm. in diameter showing portions of septa, placenta, and seeds, with necrotic areas and a rather marked displacement or abnormal arrangement of the ovules. (1) Thin plate of necrotic tissue at the advancing face of a hyperplastic outgrowth from the locule wall. (2) Necrotic pocket near an ovule. (3) Necrotic pocket in the placenta partially surrounded by a zone or whorl of hyperplastic tissue. (4) A necrotic ovule. (5) A partially necrotic ovule abnormally oriented. (6) A necrotic ovule in contact with a plate of necrotic tissue and an outgrowth from the locule wall. The ovule is somewhat changed from its normal location. Photomicrograph × 25. Unstained



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areas which may result in severe and extensive cracking. Cracking of the tubers has also been reported by Barrus and Chupp (*6, p. 125*). Murphy and McKay (*22, p. 351*) have recently reported similar blisters on tubers of the President variety in which they found evidence of cell division and a later necrosis and collapse of the cells.

The occurrence of translucent tissue in mosaic diseases or related troubles has been recorded by many observers. Bailey (*5, p. 150*), in 1892, noted dark translucent spots on tomato leaves affected with winter blight, and Orton and McKinney (*23, p. 242*) observed transitory watery blisters on tomato leaves which soon collapsed and were replaced by necrotic spots. Brandes (*7, p. 10*) found that there were water-soaked mosaic lesions on sugar cane stalks which were followed by longitudinal cracks, and Kunkel (*17, p. 3*) noted translucent spots in the very young leaves and translucent strips within the stalks of mosaic corn plants. Atanasoff (*4, p. 6*) noted translucent margins about the leaf and stem lesions of potato stipple streak, and Hungerford (*16, p. 136*) working with a similar disease, noted that the lesions were first watersoaked.

Dickson (*10, p. 48*) found that the lighter leaf areas were more translucent in a number of mosaic diseases including tomato, tobacco, potato, legumes, and raspberry and attributes this to the presence of less chlorophyll and to a reduction of intercellular space owing to the smaller, more isodiametric and closely packed cells, a condition which would facilitate the passage of light. This condition was described by Woods (*34, p. 10*) in the light areas of mosaic tobacco leaves and has also been observed by Doolittle (*11, p. 17*) in cucumber mosaic, by Rand (*26, p. 14*) in pecan rosette, and by Matsumoto (*19 p. 295*) in azuki-bean mosaic. Rand also found decreased intercellular space in the thickened, dark green leaf areas. It would seem, therefore, that the formation of translucent tissue with reduced intercellular space is rather common in the mosaic types of disease.

Necrotic spotting of the leaves and necrotic streaking of the stems seem to be of rather general occurrence among mosaic diseases. Internal necrosis, however, is not so generally recorded but has been noted by Smith and Boncquet (*32, p. 104*) in the phloem in beet curly top, by Artschwager (*2, p. 569*) in the phloem tissue in potato leaf-roll, by Robbins (*29, p. 355*) in the midrib phloem in sugar beet mosaic, and by Rankin (*27, p. 38*) in the phloem and pericycle in raspberry leaf-curl. Isolated, scattered, internal pockets or strips of necrotic tissue have been found by Matz (*20, p. 75*) in the stem parenchyma in mosaic sugar cane, by Kunkel (*17, p. 5*) in the stem parenchyma in corn mosaic, and by Güssow (*13, p. 493*), Murphy (*21, p. 79*), Barrus and Chupp (*6, p. 125*), Atanasoff (*4, pp. 7 and 11*), Artschwager (*3, p. 242*), and Murphy and McKay (*22, p. 351*) in the case of potato streak and related diseases. In sugar cane and corn, cavities were found associated with the necrotic strips and pockets.

In the tomato, Orton and McKinney (*23, p. 241*) noted internal necrotic areas in the stem, and Howitt and Stone (*14, p. 163*) state that the brown "discoloration extends deeply into the flesh of the fruit and can be traced from the epidermis along the septa to the center." Paine and Bewley (*24, p. 188*) found necrotic patches in the pith and cortex of the stem and in the petiole. Brittlebank (*8, p. 232*) also found the brownish lesions in the case of spotted wilt extending deeply into the fruit. The latter observers intimate, however, that the internal necrosis extends inward from the surface lesions, whereas in our material there was ordinarily no such direct connection between external and internal lesions.

Hypertrophy is reported by Dickson (*10, p. 34*) in the palisade parenchyma of the thickened, dark green areas in the

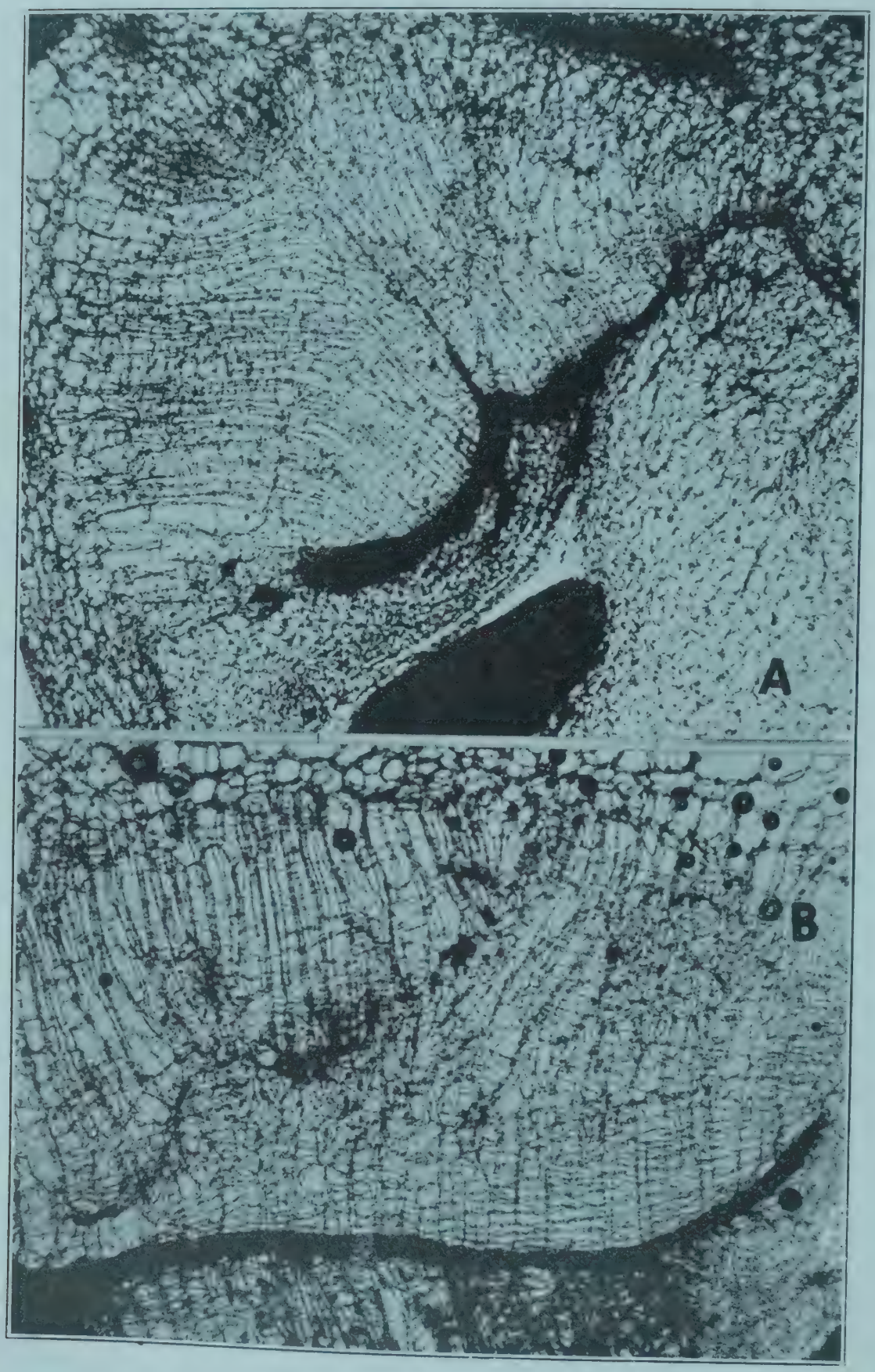
EXPLANATORY LEGEND FOR PLATE 6

A.—Cross section of pericarp of mosaic tomato ovary, 4 mm. in diameter, showing atrophy of entire thickness of wall due to early necrosis. Such areas rupture early and result in fruits such as are shown in Plate 3, C. Somewhat similar atrophy has been found in the locule walls. Photomicrograph × 95. Unstained

B.—Section through a granular fruit lesion (pl. 2, F) showing variation in thickness of pericarp due to internal necrotic planes and cell hypertrophy. At the right, inward hyperplastic growth is visible. The placental matrix bounded by a dense necrotic surface plane is pushing up against and adhering to the pericarp as a result of cell elongation; such internal pressure probably accounts for the greater firmness of the granular areas on the fruit. Photomicrograph × 33. Stained with carbol fuchsin. Fruit 12 mm. in diameter

C.—Section through a pericarp lesion showing a subepidermal necrotic plane accompanied by marked hypertrophy of the adjacent cells, and a rupture of the pericarp which extends almost to the surface. The placental matrix with its necrotic surface plane is pressing against and adhering to the pericarp. Photomicrograph × 33. Stained with carbol fuchsin. Fruit 12 mm. in diameter

D.—A necrotic region in the axial tissue of the fruit showing the collapse of the necrotic cells and the hypertrophy of the surrounding cells forming the translucent zone visible under the hand lens. Photomicrograph × 33. Stained with carbol fuchsin. Fruit 12 mm. in diameter



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leaves of mosaic tomato, petunia, legumes, and raspberry, and a similar condition was found by Doolittle (*11, p. 17*) in cucumber leaves and by Rand (*26, p. 19*) in pecan leaves. Townsend (*33, pl. 4*) showed that the spines on the leaf veins of the lower epidermis characteristic of beet curly top were due to elongation of cortical cells. Dickson (*10, p. 35*) found hypertrophied chlorenchyma cells associated with the raised green areas on mottled mosaic tomato fruits, a condition similar to the pericarp hypertrophy occasionally met with in this study.

Extensive hyperplasia or proliferation of cells was found by Allard (*1, p. 255*) in the anthers of mosaic tobacco plants. The hyperplastic development of a double layer of palisade tissue in the dark green leaf areas is reported by Hunger (*15, p. 272*) and by Dickson (*10, p. 26*) in tobacco mosaic, and Doolittle (*11, p. 18*) reports hyperplasia in connection with the projecting knobs on mosaic cucumber fruits.

Of special significance are the observations of Smith and Boncquet (*32, p. 104*) on the formation of wound-healing cells about the phloem necrosis in beet curly top, the observations of Artschwager (*2, p. 569*) upon the radial stretching or hypertrophy of the cells surrounding the necrotic areas in potato leaf-roll and the observations of Kunkel (*17, pl. 12*) upon the elongation of the parenchyma cells around the necrotic pockets or cavities in the mosaic corn stalk, leading, as he says, to incipient gall formation. These areas, Kunkel (*17, p. 11*) states, first "appear water soaked and are more turgid than the surrounding tissues," later (*17, p. 5*) "take on a slightly yellow or brown color," and "in a still more advanced stage all or a part of the cells in the pockets collapse and elongated cavities are left within the stalk." He further states (*17, p. 9*) that "the disease usually causes the host cell to enlarge . . . . Many diseased cells die and collapse. This may happen even when little or no abnormal growth has taken place. However, cells that make considerable growth die and break down earlier than cells that respond more slowly." These phenomena exhibit striking similarities with some of those observed in mosaic tomatoes, except for the unmistakable hyperplasia in the latter.

It would seem, therefore, that under certain conditions the young tomato fruit may express a range of rather striking symptoms, of which, however, some trace has been recorded in the mosaic and related diseases of other hosts. Malformation and bursting of the fruit, external and internal necrotic regions, cavities, surface blisters, translucent tissue with reduced intercellular space, elongation of cells around necrotic tissues, and hyperplasia have been previously noted. In view of the profound alterations and derangements in the normal hereditary course of development of the embryonic leaf tissues brought about by mosaic diseases in general, it is not surprising to find strikingly aberrant histological conditions in the tissues of the young, rapidly growing tomato fruit.

SUMMARY

The histological abnormalities in the young fruits of greenhouse tomato plants affected with the severe type of mosaic (streak or winter blight) were studied by means of unstained free-hand sections and stained microtome sections.

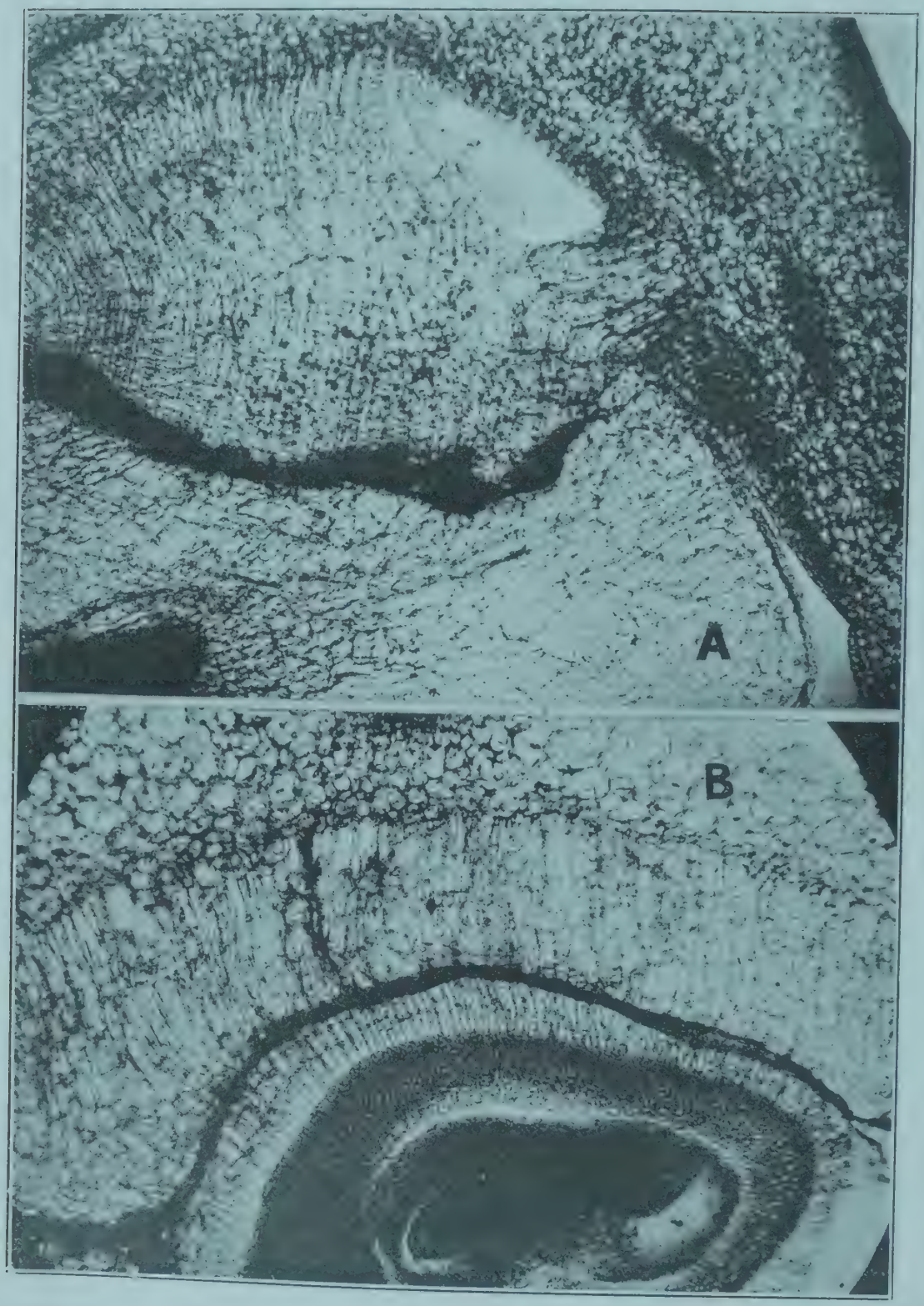
Normally the tissue of the axile placenta grows out between and around the ovules, engulfs them, and fills the locular cavity with a cellular matrix as the ovary enlarges. This placental matrix touches, but remains free from the carpellary walls and the seed coats.

Many of the mosaic fruits are characterized by brown necrotic surface dis-

EXPLANATORY LEGEND FOR PLATE 7

A.—Cross section of an outer angle of a locule in fruit about 12 mm. in diameter with a double intumescence invading and crushing the placental matrix. The normal thickness of the pericarp is shown at lower left corner. Two large hyperplastic intumescences, one from the inner surface of the pericarp, one from the radial wall, have grown inward and met each other, after which the direction of growth was somewhat altered in each case. There is a thin plane of crushed necrotic tissue at the advancing face of the growths which have almost reached the ovule shown at the bottom of the figure. These intumescences represent an abnormal hyperplastic growth 2 mm. in length. This constitutes an adhesion between the locule walls and the placenta. Photomicrograph × 33. Unstained

B.—Section of a hyperplastic growth on the inner surface of the pericarp pushing into the placental tissue. The palisade columns of rectangular, thin-walled cells indicate the direction of growth and the muriform arrangement with the reduced intercellular space explains the translucent appearance of such tissue. The advancing face impinges upon a thin plate of crushed necrotic cells and within the hyperplastic tissue a secondary necrotic plane is developing, apparently as a result of increased pressure from the tissues between this point and the base of the intumescence. The impression is gained from this and similar sections that groups of basal cells often begin to divide too rapidly for the more distal cells and crowd into the latter from the rear. As in A, this constitutes an abnormal adhesion between locule wall and placenta, since the tissues do not usually separate along the necrotic planes. Photomicrograph × 33. Unstained



(For explanatory legend see p. 887)

coloration, by raised brownish translucent blisters, or by sunken necrotic lesions exhibiting a great variety of shapes and sizes and arranged in peculiar patterns. Such lesions on very young fruits result in great malformation.

Deep-seated necrosis in the pericarp and subsequent atrophy or collapse of the affected tissues result in extensive rupture of the fruit-wall and cracking of the fruit. Hypertrophic thickening of the pericarp was found.

Internal necrotic regions, surrounded by zones of translucent tissue, occur throughout the fruit, particularly about the periphery of the placental matrix. Cavities are often associated with these necrotic regions.

Abnormal adhesions between the ovules and the placental matrix and between the latter and the lining of the locular cavity are of rather frequent occurrence at the necrotic planes.

Ovules may be disarranged or abnormally oriented and may be retarded or atrophied. Seeds may show brown spots under the seed coat. The placental matrix may develop abnormally.

The epidermal blisters are caused by cushions of muriform hyperplastic tissue which push up under the necrotic epidermis. The translucent appearance of this tissue is caused by the reduced intercellular space.

The internal necrotic regions are usually isolated strips, pockets, or plates of crushed brown tissue surrounded or accompanied by zones of radially elongated cells or zones of hyperplastic tissue composed of parallel columns of meristematic cells growing in toward the necrotic region. Hyperplasia is most marked in cases where epithelial tissues are involved.

Rather well developed intumescences grow inward from the locular walls and invade the placental matrix, resulting in abnormal tissue fusions or adhesions.

Necrosis and cell hypertrophy within the seed and abnormal cross walls in the palisade epidermal cells of the seed coat were found.

Apparently hypertrophy and hyperplasia occur only in association with necrosis and are responses to the latter.

A review of the literature on mosaic and related diseases shows that certain of these phenomena have been previously observed.

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EXPLANATORY LEGEND FOR PLATE 8

A.—Cross section through outer angle of a locule showing a large intumescence from the locule wall and a smaller one from the inner surface of the pericarp pushing a plane of crushed necrotic tissue down into the placental parenchyma. The normal thickness of the pericarp is shown at the upper right corner and the normal smooth inner surface at either side of the smaller intumescence. In the lower right corner may be seen the normal outer surface of the placental parenchyma or matrix that fills the locular cavity and in the lower left corner is an ovule. Photomicrograph × 27. Unstained

B.—Section through a hyperplastic growth from a locule wall (above) which has pressed a plane of crushed, necrotic tissue against a seed with the resultant production of a few abnormal cross walls in the palisade epidermal cells of the latter. This constitutes an adhesion between the locule wall and the seed coat. Photomicrograph × 26. Unstained

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FEEDING CHLORINATED MILK TO THE ALBINO RAT[1]

By J. W. READ, *Head of the Department of Agricultural Chemistry, Arkansas Agricultural College,* and HARRISON HALE, *Head of the Department of Chemistry, University of Arkansas*

INTRODUCTION

Considerable interest has been shown recently in the feasibility of using available chlorine, particularly in the form of hypochlorites, as a germicide in milk and milk products. Many dairymen are now using hypochlorite solutions sold under such trade names as B. K. (Bacteria Kill) to cleanse and disinfect their utensils, and it is also known that some of these have ventured to add these preparations to their milk with a view to preservation. Because of this practice, the question naturally arose as to what harmful effects, if any, might be produced by the consumption of milk which had been treated with given amounts of available chlorine.

The results reported in this paper cover certain experiments conducted in 1919–20 on white rats to determine whether or not there would be any apparent ill effects on the rats by the consumption of liberal amounts of whole milk treated with definite concentrations of active chlorine either as chlorine water or in the form of sodium and calcium hypochlorites. These studies constituted one phase of an investigation undertaken to determine the efficiency of available chlorine as a germicide in milk and milk products.

EXPERIMENTAL PROCEDURE

ARSENIOUS ACID.—Baker and Adamson's C. P. arsenious acid was still further purified by recrystallization three times from hot water. It was then sublimed three times. Carefully dried crystals from the third sublimation were used to prepare the standard arsenious acid solution.

POTASSIUM IODIDE.—Merck's Reagent, neutral potassium iodide, was used in a 20 per cent aqueous solution for the iodometric titration of chlorine.

STARCH SOLUTION.—This solution was prepared as directed in Treadwell and Hall's Analytical Chemistry.[2] Two cubic centimeters of the starch solution were used for each titration.

PREPARATION OF THE STANDARD ARSENIOUS ACID SOLUTION.—This solution was prepared in accordance with the recommendation of Washburn,[3] using sodium hydroxide and phosphoric acid solutions of known strength to insure a better regulation of the hydrogen-ion concentration during the titrations and to obtain more sensitive end points. The sodium hydroxide was purified by alcohol, and the phosphoric acid was Merck's Reagent, 85 per cent, sp. gr. 1.710.

IODOMETRIC TITRATION OF CHLORINE.—An accurately prepared one-tenth normal arsenious acid solution was used to titrate the iodine liberated from 15 c. c. of 20 per cent potassium iodide solution by a definite volume of dilute hypochlorite solution or chlorine water. In the case of the hypochlorite solutions, satisfactory end points were not readily obtained if the concentration of the active chlorine was much greater than 6 to 12 mgm. per 100 c. c. of solution to be titrated. The plan followed to obtain this strength included the dilution of 12.5 c. c. of the stock hypochlorite solution to 500 c. c., whereupon 10 c. c. of the dilute solution (representing 0.25 c. c. of the stock preparation) were used for the titration. It was, however, not necessary to follow the above procedure of diluting when titrating the chlorine water, in which case 1 c. c. of the stock solution was diluted to 100 c. c. at the time of titration.

EXPERIMENTAL ANIMALS.—The diet of the experimental animals consisted of an intimate mixture of equal parts of finely ground whole wheat and finely ground yellow corn, a liberal quantity of treated whole milk, and 2 per cent of a salt mixture consisting of equal weights of sodium chloride and calcium carbonate. Four animals, two females and two males, were used in

[1] Received for publication July 15, 1924; issued June, 1925. Research Paper No. 9, Journal Series, University of Arkansas.

[2] TREADWELL, F. P. ANALYTICAL CHEMISTRY, TR. BY W. P. HALL. Ed. 3. 2: 652. 1911.

[3] WASHBURN. E. W. THE THEORY AND PRACTICE OF THE IODOMETRIC DETERMINATION OF ARSENIOUS ACID. Jour. Amer. Chem. Soc. 30: 31–46, illus. 1908.

each experiment. The rations for the various groups of experimental animals differed only in the proportion of active chlorine added to the whole fresh milk given the animals each day in amounts varying from 1 part of chlorine in 3,000 parts of milk to 1 part of chlorine in 15,000 parts of milk. The experimental animals were weighed every 20 to 25 days, and the experiments extended over a period of 10 months. Birth records were carefully observed, but the intervals between weighings were too long to permit of the usual type of curve showing growth and reproduction for the females. Their weight and reproduction records are, therefore, placed in tabular form, and probably serve the purpose of this paper equally as well. The growth of the males is shown in Figures 1, 2, and 3. While no quantitative consumption data were kept, the animals whose experimental records are given in this paper consumed liberal quantities of the treated milk from day to day, and the variations in milk consumption among the different experimental groups are considered to be of little or no consequence.

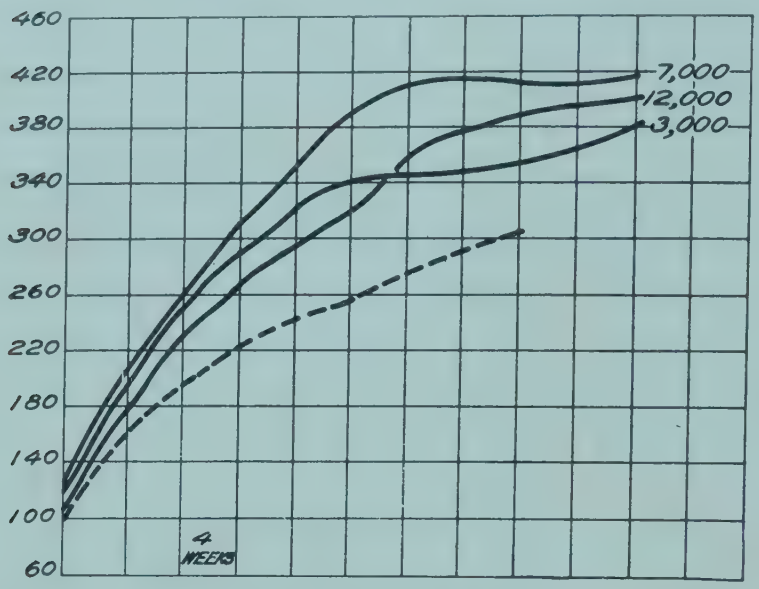

FIG. 1.—The above curves represent the growth of males receiving 1 part of active chlorine (in the form of calcium hypochlorite) in 3,000, 7,000, and 12,000 parts of fresh whole milk. The rest of the diet consisted of an intimate mixture of equal parts of whole wheat and whole corn, both finely ground, plus 1 per cent each of sodium chloride and calcium carbonate. The dotted curve represents normal growth

DISCUSSION

In their patent relating to a process of treating milk, cream, butter, etc., with hypochlorous acid gas under pressure, Fox and Bates[4] claim that "the pathogenic germs of milk will be destroyed without effecting the enzymes in the milk and without leaving any deleterious products in the milk or otherwise injuriously affecting it." They also state that cream so treated produces a higher grade of butter, as it can be churned at a lower temperature and any foul odors resulting from bacterial decomposition are removed and the injurious organisms destroyed.

Rupp[5] describes a test for the detection of hypochlorites and chloramins in milk and cream by means of potassium iodide and hydrochloric acid. He claims that 1 part of chlorine in 50,000 parts of milk or cream can be detected by this method; also that milk kept in the ice box for 24 and 48 hours and milk pastuerized after the addition of hypochlorites at 145° F.

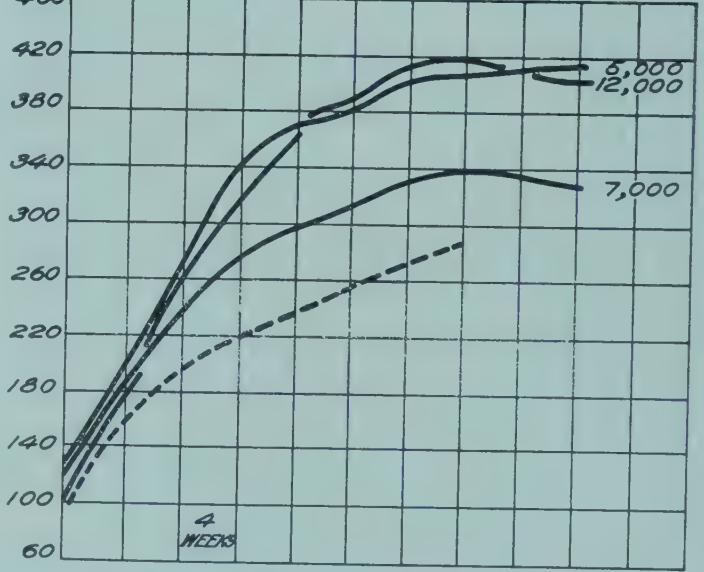

FIG. 2.—This figure shows the growth curves of males receiving 1 part of active chlorine (in the form of sodium hypochlorite) in 5,000 7,000, and 12,000 parts of fresh whole milk. The rest of the diet consisted of an intimate mixture of equal parts of whole wheat and whole corn, both finely ground, plus 1 per cent each of sodium chloride and calcium carbonate. The dotted curve represents normal growth

[4] FOX, A. O., and BATES, R. R. PROCESS OF TREATING LIQUIDS. U. S. Patent No. 1,114,875. U. S. Patent Office, Off. Gaz. 207: 999, illus. 1914.

[5] RUPP, P. THE DETECTION OF HYPOCHLORITES AND CHLORAMINS IN MILK AND CREAM. U. S. Dept. Agr. Bul. 1114, 5 p. 1922.

for 30 minutes will still give the reaction.

Hale and Bleecker [6] state that active chlorine does act as a germicide in milk and in ice cream with a reduction in the number of bacteria proportional in general to the amount of active chlorine present, but they are not willing to recommend chlorine for treating market milk on the basis of their experimental results covering its germicidal value. The experiments presented in their paper were conducted simultaneously with those presented in this paper, and the concentration of chlorine in the milks used by Hale and Bleecker were controlled by the standard arsenious acid solution prepared especially for obtaining the data given in this paper. The results presented in these two papers are, therefore, entirely comparable from the standpoint of the concentration of active chlorine in the treated milk.

The experimental rats came from a standardized stock. Less than one-third of the animals placed on experiment have been reported in this paper, inasmuch as those on the higher concentrations of chlorine fared equally as well as did those on the weaker concentrations and also the controls. The calcium and sodium hypochlorites imparted considerable flavor in the 1:3,000 and 1:5,000 concentrations, but this did not prevent the animals from consuming liberal quantities of the treated milk; and it will be observed from the table and Figures 1, 2, and 3 that the growth and reproduction records of these animals are as satisfactory in every respect as are those of the control animals.

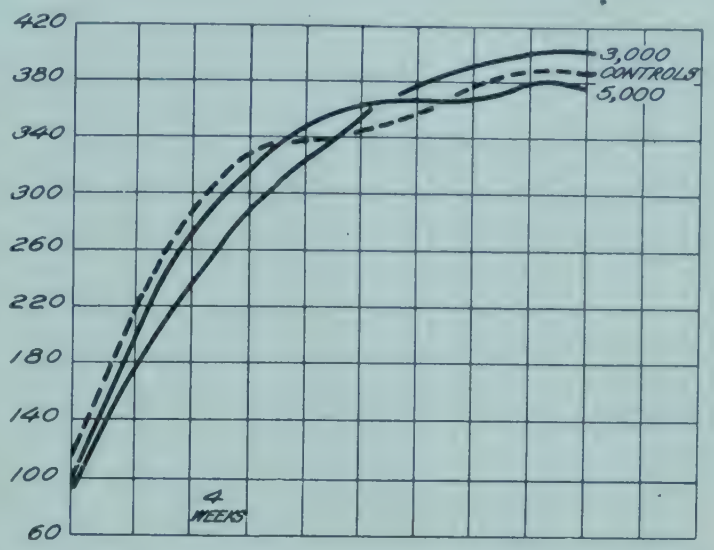

FIG. 3.—The curves in this figure show the growth of male rats receiving 1 part of active chlorine (in the form of chlorine water) in 3,000 and 5,000 parts of whole fresh milk. The rest of the diet consisted of an intimate mixture of equal parts of whole wheat and whole corn, both finely ground, plus 1 per cent each of sodium chloride and calcium carbonate. The dotted curve represents the growth of the control animals receiving the untreated milk

SUMMARY

The experiments reported in this paper indicate that the feeding of milk treated with active chlorine in the concentrations studied produces no harmful effects upon the white rat as far as has been observed from investigations of this nature extending over a period of 10 months. On the other hand, this statement is not a recommendation for the treatment of milk with active chlorine.

[6] HALE, H., and BLEECKER, W. L. ACTIVE CHLORINE AS A GERMICIDE FOR MILK AND MILK PRODUCTS. Jour. Agr. Research (1923) 26: 375–382, illus. 1924.

Growth and reproduction records—female

I.—Controls Received Untreated Milk

Date	Weight of dam	Date of birth	Number of young	Age of young in days	Average weight of young	Normal weight of young [a]	Ratio of actual weight to normal weight
Female A:							
Sept. 9	155	Aug. 16	5	24	34.0	23.7	1.4
Sept. 22	134	do	5	36	52.4	36.2	1.4
Oct. 1 [b]		do	5	45	74.2	52.5	1.4
Female B:							
Oct. 29	253	Sept. 9	8	50	71.3	59.4	1.2
Dec. 22	228	Nov. 27	11	25	32.7	24.7	1.3
Jan. 16	272	do	11	50	90.7	59.4	1.5
Feb. 7	242	do	11	72	144.0	106.2	1.4
Feb. 27	243	Jan. 16	5	42	47.0	46.1	1.0

II.—Chlorine Water

1 part active chlorine to 3,000 parts milk:							
Sept. 22	190	Sept. 5	6	17	25.8	17.7	1.5
Oct. 9	215	do	6	34	53.3	34.9	1.5
Nov. 29	207	Oct. 30	6	30	41.3	30.1	1.4
Jan. 25	185	Dec. 24	8	32	42.0	32.4	1.3
1 part active chlorine to 5,000 parts milk:							
Sept. 22	149	Sept. 4	8	18	18.4	18.4	1.0
Nov. 29	203	Oct. 24	6	36	57.0	37.6	1.5
Jan. 19	186	Dec. 17	8	33	45.6	33.6	1.3
Mar. 2	193	Feb. 13	6	34	39.6	34.9	1.1
Apr. 29	202	Apr. 3	6	26	30.8	25.7	1.2

III.—Sodium Hypochlorite

1 part active chlorine to 5,000 parts milk:							
Sept. 9	193	Aug. 20	5	20	26.8	20.1	1.3
Sept. 22	191	do	5	33	46.2	33.6	1.4
Oct. 1	199	do	5	42	66.8	46.2	1.4
Oct. 28	194	Oct. 6	9	22	24.4	21.8	1.1
Nov. 15	234	do	9	40	55.9	43.1	1.3
Dec. 23	217	Nov. 30	5	23	40.6	22.7	1.8
1 part active chlorine to 7,000 parts milk:							
Sept. 9	170	Aug. 15	10	25	29.4	24.7	1.2
Sept. 22	184	do	10	38	49.4	40.3	1.3
Nov. 15	212	Oct. 17	10	28	47.8	27.8	1.7
Jan. 7	253	Dec. 7	9	31	42.6	31.2	1.4
Feb. 27	279	Jan. 29	9	29	38.2	28.9	1.3

IV.—Calcium Hypochlorite

1 part active chlorine to 3,000 parts milk:							
Sept. 8	192	June 25	4	75	137.2	113.8	1.2
Sept. 22	240	Aug. 20	4	33	56.0	33.6	1.7
Oct. 25	195	Sept. 23	8	32	49.2	32.4	1.5
Nov. 25	224	Nov. 19	6	10	19.5	13.3	1.5
Dec. 22	212	do	6	33	64.6	33.6	1.9
1 part active chlorine to 7,000 parts milk:							
Sept. 8	188	Aug. 7	8	32	41.1	32.4	1.3
Sept. 22	202	do	8	46	64.4	52.5	1.2
Oct. 25	247	Oct. 11	5	14	26.5	15.7	1.7
Nov. 4	247	do	5	24	51.0	23.7	2.1
Nov. 29	246	Nov. 9	8	20	30.5	20.1	1.5
Dec. 22	255	do	8	43	80.1	47.7	1.7
Feb. 5	234	Jan. 10	8	26	37.4	25.7	1.5

[a] The figures given in this column represent the average combined weight of male and female rats at a given age in days, according to Donaldson's original tables. It is now recognized that these values represent moderate curves of growth.

[b] Ill, removed.

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THE POSSIBILITY OF SEX CONTROL BY ARTIFICIAL INSEMINATION WITH CENTRIFUGED SPERMATOZOA[1]

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INTRODUCTION

The phenomenon of sex is one of the most conspicuous features of living things, both plant and animal. Differentiation of the reproductive organs into two sorts, the one called male and the other female, is almost the universal rule among all but the very simplest of both plants and animals. However, a great variety of arrangements of the male and the female reproductive organs exists in the different groups of the plant and animal kingdoms. Thus the great majority of the higher plants (spermatophytes) and many of the lower forms of animals have both the male and the female reproductive organs in the same individual. On the other hand, some of the higher plants (such as hemp, mulberry, date palm, and many others), and the great majority of the higher animals (including practically all of the vertebrates and arthropods and many of the lower animals), have the male reproductive organs in one individual and the female organs in another.

Sex is popularly considered as a feature of the animal kingdom alone and is scarcely associated with plants. Although this idea of the existence of sex is biologically wrong, it is economically sound because all but a few of the plants of economic importance are monoecious, while all of the mammals, birds, and insects are normally dioecious, and there is frequently a considerable difference in the value of the two sexes of economically important animals.

This article considers the problem of sex control in the light of what is now known about the mechanism of sex determination and gives a detailed report of an experiment carried out at the Wisconsin Agricultural Experiment Station to test the possibility of controlling sex by artificial insemination with centrifuged spermatozoa. This experiment resulted in the discovery of a number of interesting facts, especially in regard to the success and methods of artificial insemination and in regard to the variations in the size of the spermatozoa. It did not succeed in its primary purpose of controlling the sex of the offspring thus produced, but the results of the experiment do throw some light upon the possibility of sex control by such a method and are therefore presented here.

DEFINITIONS

The term "sex determination" is used here to mean the process which determines that any given individual shall be of a definite sex—that is, male, female, or some grade of hermaphrodite. It is an observed fact and must have a mechanism upon which it rests whether or not that mechanism is as yet clearly demonstrated. The term "sex control," on the other hand, is used to mean the modification of the process of sex determination so that the offspring will be of the sex which the breeder desires. The control of sex among the higher nonparthenogenetic animals has not yet been completely demonstrated, and it is quite possible that even a complete knowledge of the mechanism of sex determination will not lead to any successful method of sex control—that is, the problem of sex determination has a solution which may be completely demonstrated at some future time and probably has already been found in the chromosome theory of sex determination or some modification of it, but the problem of finding a practical method of sex control need not necessarily have any solution.

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[2] Now Animal Husbandman, Texas Agricultural Experiment Station.

REVIEW OF LITERATURE

THEORIES RELATING TO THE DETERMINATION OF SEX

The phenomena of sex play such a large part in human life that they have attracted the attention of investigators and philosophers since the earliest time, and it is not surprising to learn that there have been many theories proposed to account for the determination of sex. Geddes and Thomson (*11*)[3] estimate that there were as many as 500 of these theories at the beginning of the nineteenth century and state that the number has continued to increase. Undoubtedly the increase had not stopped when they made that statement, for the theory which is most widely accepted by scientists at present, the chromosome theory of sex determination, was not suggested by McClung (*28*) until 1902, a year after the revised version of Geddes and Thomson's book was published. The only one of all these theories that has succeeded in winning any considerable amount of both popular belief and experimental support is the chromosome theory of sex determination. The experimental work upon which this paper is based rests upon the chromosome theory as a working hypothesis.

According to that theory the sex of the individual which results from the union of the egg and spermatozoon is determined by whether it contains two X-chromosomes or an X-chromosome and a Y-chromosome. Thus, in the mammals, females contain two X-chromosomes in each of their cells, and each of the egg cells which they produce contains one X-chromosome. Males contain an X-chromosome and a Y-chromosome in each of their cells, and half of the spermatozoa which they produce contain an X-chromosome and the other half of the spermatozoa contain a Y-chromosome. If the egg cell is fertilized by an X-bearing spermatozoon, the resulting individual will contain two X-chromosomes in its cells and will be a female. If the egg cell is fertilized by a Y-bearing spermatozoon the resulting individual will contain an X-chromosome and a Y-chromosome in its cells and will be a male. Sex in birds and in the Lepidoptera is determined in the same way except that the sexes are reversed with respect to chromosome composition. Recent discoveries (*3*, *4*, *5*, *12*, *37*) have made it seem likely that the X-chromosome itself does not determine sex directly, but that its action is due to sex-modifying factors, of which the X-chromosome carries a disproportionately large and unbalanced number but of which the other chromosomes carry a few. This modification of the originally simple chromosome theory of sex determination is of importance chiefly in that it offers an explanation of sex intergrades and has a bearing on the question of the absolute irreversibility of sex.

The proof of the existence of a causal relation between the sex chromosomes and sex determination is so strong that biologists almost universally concede that the sex chromosomes are the mechanism which ordinarily determines sex. However, it may not necessarily follow from this that sex can not be reversed by any unusual combination whatever of the other forces which may have an influence upon sex. Indeed some experiments (*9*, *10*, *18*, *34*, *35*, *36*) have been interpreted as showing that it is occasionally possible to cause an individual to change its sex. There is still much confusion on this point, and until that confusion is cleared up the possibility of complete functional sex reversal can not be disproved, at least among animals like the birds and amphibians in which the two sexes do not differ greatly with regard to the gross anatomy of their organs of reproduction. However, very much of this confusion is due to a failure to distinguish clearly between the primary differentiation into two sexes and the secondary effects produced by the hormone action of the ovary or testis.

Several studies have given results which conform to the chromosome theory of sex determination but indicate that it may be possible to control sex by controlling the chromosome distribution. One of these is the series of studies (*31*, *32*, *33*) of chromosome behavior in groups like the aphids, which reproduce sometimes parthenogenetically and sometimes sexually. In some species the same aphid may produce males or females, or other parthenogenetic females like herself (*38*). Apparently there is something here governing the chromosome behavior, but what it is or to what extent it is "internal" and to what extent it is a result of the environment (and therefore possibly controllable) is not known. Riddle's work (*34*, *35*, *36*) with sex in pigeons seems to demand the same explanation. He claims to have controlled sex in pigeons by the factor of reproductive overwork—that is, the later eggs from females which had been forced to

[3] Reference is made by number (italic) to "Literature cited," p. 912.

lay a very large number of eggs produced a much higher proportion of females than did the earlier ones. Jull (*21*, *22*), working with the domestic fowl, confirmed Riddle's results as to the relation between reproductive overwork and the sex of offspring, but by making use of a sex-linked character in his breeding experiments Jull showed that all the males and females had the chromosome composition normal to their sex. This is added evidence in favor of the chromosome theory of sex determination, and the problem in the work of Riddle and of Jull becomes the discovery of what factor in the condition of reproductive overwork governs the segregation of the sex-determining chromosome into the ovum or the polar body at the reduction division. Hays (*16*) obtained similar results with rabbits.

There have been two notable successful attempts to establish, by selective breeding, strains which produce either an excess of females or an excess of males. The first of these was by Moenkhaus (*30*) working with Dorsophila. He never succeeded in getting a strain which would produce a constant significant excess of males but did succeed almost from the start in getting a strain which averaged about 75 males to 100 females. Warren (*39*), who repeated the same kind of experiment on a different strain of Drosophila without success, showed that the results of Moenkhaus harmonized perfectly with the idea that he was dealing with a strain which carried a sex-linked lethal factor, and since more than 20 such sex-linked lethal factors have already been found in *Drosophila melanogaster* this case seems to support rather than to disprove the chromosome theory of sex determination. The second successful attempt to establish excess-male and excess-female lines by selective breeding was that made by King (*23*), using albino rats. King's results also conform fairly well to the hypothesis that her initial stock contained lethal factors. The results in the low male line fit the hypothesis that she was dealing with a strain containing a sex-linked lethal factor very well, except for the fact that the inbred females when bred to stock males produced a somewhat larger proportion of males than when bred to full brothers. The results in the high male line fit even better the hypothesis that she was dealing with a strain containing a sex-linked factor which, when homozygous, is lethal for females but has no effect upon males. (A similar factor has been reported (*2*) in Drosophila.)

RELATION OF THE CHROMOSOME THEORY OF SEX DETERMINATION TO THE PROBLEM OF SEX CONTROL

The existence of complete functional sex reversal is still in question even when considering birds and amphibians. Moreover, those cases which have been reported as proving such sex reversal in birds (*9*, *10*, *35*, *36*) do not offer proof as to how such sex reversal might be controlled by the breeder. Furthermore, Lillie's work (*24*, *25*, *26*) on the freemartin has made it appear that complete sex reversal among the mammals is still more unlikely. Aside from the possibility of sex reversal, the problem of sex control may be approached from two sides—either with the idea of destroying the fertilized eggs which are of the undesired sex, or with the idea of preventing the formation of such fertilized eggs.

The work of Riddle (*34*), Jull (*22*), and Hays (*16*) may furnish a clue to some method of sex control, but as yet the underlying principles governing the partial success which they obtained in the control of sex have not been clearly demonstrated and have not been shown to be as completely susceptible to human control as would be desirable in a practical method of sex control. Killing or rendering impotent the spermatozoa which would produce the undesired sex seems to be a simpler method of attack, because treatment of the spermatozoa with a poisonous chemical agent could be in vitro, and the female could afterward be fertilized by artificial insemination.

Separation of male-producing and female-producing spermatozoa by physical means seems more possible than by chemical means. The physical differences upon which separation seems most possible are differences in motility and differences in size. A difference in motility might itself be due to a difference in size which would result in the smaller spermatozoa offering less resistance to forward motion through the liquid by which they are surrounded. This is a possible explanation for the normal excess of males born among most species of mammals. In order to take advantage of differences in motility it would be necessary to keep the spermatozoa oriented in one direction. Possibly this might be done by means of an electric current, but, so far as the author knows, this has not been tried. A physical separation of the spermatozoa upon the basis of their size difference hardly seems possible by any filtering or straining method because of their smallness and delicacy. However, separation of the two sizes

by means of centrifugal force exerted upon them while they are immersed in a liquid of very nearly the same density as themselves is possible theoretically, and since some preliminary experiments along that line had indicated that success might actually be obtained in this way the experiments which are reported in this paper were designed to test that point.

DIMORPHISM IN SIZE OF SPERMATOZOA

The existence of two distinct sizes of spermatozoa in many animals is to be expected, particularly in those in which the Y-chromosome is absent or very much smaller than the X-chromosome. The head of the spermatozoon is composed almost entirely of chromatin, and therefore the spermatozoa which receive an X-chromosome should have larger heads than those which receive the Y-chromosome except in cases where the Y-chromosome is as large as or larger than the X-chromosome. This has actually been found in a number of species of vertebrates and insects (*43*, *44*, *45*, *46*, *47*). Considering that the size of the spermatozoon head depends upon three dimensions and possibly may vary in any one or all of these dimensions more or less independently, it is remarkable that the results obtained show rather uniformly two size groups in nearly equal numbers when only the head lengths were measured. However, each species has a rather characteristic shape for its spermatozoon heads and the length of the head may be a fairly reliable index to its total size. Two classes of spermatozoa have been found, the differences between their mean head lengths approximating very closely in most of the species the theoretical differences calculated from drawings of the chromosomes as seen during maturation divisions of the germ cells.

EXPERIMENTAL PROCEDURE

FACTORS INVOLVED IN THE PROBLEM

In undertaking experimental work involving the separation of spermatozoa by centrifugal force, several factors had to be considered. If there is no difference in the density of the two sizes of spermatozoa the larger ones will offer less resistance than the smaller ones, per unit of weight, to locomotion through a surrounding liquid, whether the specific gravity of the liquid be greater or less than their own, because the volumes of the larger ones (and therefore their weights, upon which depend the forces tending to move them through the liquid) are proportionately greater per unit of surface or cross section (upon which depends the resistance which they offer to locomotion through the liquid). If the specific gravity of the liquid were very much more or very much less than that of the spermatozoa themselves, a very little centrifuging would cause both classes of spermatozoa to go either to the inside or to the outside of the centrifuge tube together, but if the specific gravity of the liquid were very nearly that of the spermatozoa their progress during the centrifuging would be very much slower, and if the right amount of centrifugal force were used for the right length of time a higher percentage of the large than of the small spermatozoa would reach the inside or the outside of the centrifuge tube, according to whether the specific gravity of the liquid were more or less than that of the spermatozoa. If, however, the two sizes of spermatozoa differ in specific gravity, a thing for which there is no proof and which there are no a priori reasons to expect, but which might nevertheless be true because the chromatin of the sex chromosome might be of a different specific gravity from that of the other chromosomes, the problem becomes somewhat different. If, in this case, the specific gravity of the liquid could be made intermediate to that of the two classes of spermatozoa, it would be possible to effect a more complete separation than under any other conditions, for prolonged centrifuging should result in one class going to the inside and the other to the outside of the centrifuge tube. If the larger ones have the greater specific gravity, they would move through the liquid still more rapidly than if there were no difference in specific gravity, since in addition to having less resistance per unit volume they would also have a greater weight per unit volume than the smaller ones. If the smaller ones have the greater specific gravity, this would counterbalance their greater resistance per unit volume, and the only means of separating them by centrifugal force would be to adjust the specific gravity of the liquid to a value intermediate to that of the large and of the small spermatozoa.

Thus, there are at least six important factors which influence the success of the centrifugal method of separation of the male-producing from the female-producing spermatozoa. The first is the difference in size of the two classes, a factor which is not under control at

all and which is of cardinal importance. The second factor of success is the difference, if any, in the specific gravity of the two classes of spermatozoa, which also is not under control and moreover is not readily ascertainable. The third and fourth factors are the specific gravity and viscosity of the surrounding liquid. These could be controlled within limits by the addition of water or of certain solutes, provided this was not carried so far as to injure the spermatozoa. A more serious difficulty in the way of controlling the specific gravity and viscosity of the liquid lies in the fact that the liquid as recovered from the vagina and uterus of the female by a catheter varies so greatly in those properties that it would be necessary to determine them anew for every sample. The fifth and sixth factors are the amount of centrifugal force used and the length of time for which it is applied. These are quite within the control of the experimenter, but he has no way to determine the correct time or the correct force to use except by trial. Moreover he can only know whether he has succeeded by a microscopic examination (measurement) of the spermatozoa in samples from the inside and from the outside of the centrifuge, which would require a good many hours of continuous work and practically could not be carried out until the spermatozoa were too old to be viable, so that no advantage could be taken of the knowledge. It will readily be seen that the difficulties in the way of finding and maintaining the correct technique are very many and very serious, but nevertheless they did not seem insuperable.[4]

MATERIAL

The rabbits used were of mixed stock, small or medium in size, derived largely from such varieties as the Black Dutch, New Zealand Red, Himalayan, and ordinary albinos and other colors of mixed ancestry. The advantages of the rabbit as material for work of this sort are that it is of very convenient size for operation and for artificial insemination; that the period of gestation is very short and the size of litter is large, thus permitting the production of large numbers in a very short time because the young can be killed at birth if their sex is the only point on which information is wanted; that they breed all the year (to some extent, although more readily at some seasons); and finally, that they are rather cheaply maintained, and since they are mammals it is reasonable to suppose that principles learned from them would apply also to other domesticated mammals or to man. The disadvantages of using the rabbit are that the females are quite subject to infection of the uterus and Fallopian tubes by pus-forming bacteria and the difference in size between the X-chromosome and Y-chromosome is not very great(*1*).

The material from swine was obtained from three different Poland-China boars from the University of Wisconsin herd. Two of these were mature and one was about 10 months old. The two mature ones were castrated and the testicles, in the one case immediately and in the other case after immersion for about three hours in water at about 25° to 35° C., were opened with a knife and a milky fluid which oozed out from the many cut ends of the epididymis was collected by means of a catheter and a rubber bulb. The young boar was allowed to copulate with a sow and the fluid which could be recovered from the sow immediately afterward with a catheter and rubber bulb was used for the microscopic studies. Swine are too slow breeding and too expensive to maintain for the carrying out of the actual breeding experiment unless there should be unusually good reasons to expect success with them. The chromosome composition of swine as reported by Wodsedalek (*43*) is such as to give more reason to expect success in the centrifugal separation of the two classes of spermatozoa in this animal than in most, or perhaps than in any other which has yet been studied. It should be added that Hance (*14*) could not confirm Wodsedalek's results. According to Wodsedalek, the male-producing spermatoza contain 8 and the female-producing spermatozoa 10 chromosomes, and, as nearly as can be judged from his drawings, the 2 chromosomes which together behave as a single X-chromosome constitute 20 to 30 per cent of the volume of the chromatin in the spermatozoa which contain 10 chromosomes—that is, the volume of the male-producing spermatozoon might be expected to be 70 to 80 per

[4] The work was done in the laboratories of the department of genetics at the University of Wisconsin. It was originally planned by and was continually under the direction of L. J. Cole. Some preliminary work by Sarah V. H. Jones and L. J. Bachhuber had indicated that mechanical separation of the male-producing and female-producing spermatozoa might be possible by means of centrifugal force, and therefore that sex control would be possible by artificially inseminating the females with only the desired kind of spermatozoa. On the strength of this preliminary showing a detailed test of this possibility was made, using rabbits for the experimental animals but also making a few tests on semen from swine. The microscopic work was done with the advice and assistance of M. F. Guyer of the zoology department.

cent of that of the female-producing spermatozoon. His measurements of head lengths of mature spermatozoa also show a very distinct dimorphism, the smaller mode being 82.5 per cent of that of the larger one, which is a very close check with the calculated difference when it is considered that he was measuring only one dimension, whereas volume would depend upon three dimensions. The main facts as to the suitability of the animals used are contained in the statement that rabbits are excellent material in every respect but one—their chromosome composition—and that swine are excellent material chiefly in that one respect alone.

PLAN OF EXPERIMENT

The plan of the breeding experiment was to secure normal rabbit spermatoza immersed in the liquid which naturally surrounds them in a normal copulation, then to subject them to centrifugal treatment, then to inject various portions of the centrifuged liquid into the vagina or uterus of the female rabbits which were in heat, and finally to observe the sexes of the offspring resulting from these different kinds of artificial insemination.

FEMALE BEHAVIOR

It was necessary to know whether a female was in heat, and since there is no more certain sign of oestrus in the doe rabbit than her willingness to copulate with the buck, a number of sterile males were provided to act as teasers or test males to determine whether a female was in heat and yet to avoid having her become pregnant to a natural service. There are other indications of oestrus, more or less reliable, such as the degree of congestion or swelling of the vulva, mounting the other females, etc., but none of these indications was completely reliable for all the female rabbits. Each female had a type of behavior, when in heat, to which she adhered rather consistently, and it was possible after becoming acquainted with her individual record to predict rather accurately whether she was in heat without actually placing her with the male. However, the method of placing the females with the test males was always relied upon as being more accurate and almost as convenient as any other. If the doe was in heat, she usually accepted the buck almost immediately when placed with him, and if she did not accept him in the first minute or two there was very little probability of her accepting him at all, no matter how long he was allowed to tease her; but there were exceptions to this, and very rarely a doe that had refused one buck for as much as 10 or 15 minutes would accept another almost immediately when placed with him.

TEST MALES

The test males were made sterile by the operation of vasectomy, which is very easily performed on the rabbit. The test males varied in their behavior and in the ardency of their sexual desire, but so did the normal males, and although no method of making a measurement of this feature was devised, close observation leads to the conviction that the males which were not vasectomized until they were sexually mature were no less ardent and no less active sexually than the perfectly normal males. Whether the males vasectomized before they were sexually mature were affected by the operation at all remains somewhat uncertain. Some of them did develop normally and became quite ardent, but there were others which always remained rather weakly and never became ardent enough to be satisfactory test males. However, this was also true of some of the untreated males. There was nothing in this work to prove or even to indicate very strongly that the operation of vasectomy produces any psychic changes (*40*).

RECOVERY OF SEMEN

Ejaculation can not be induced in the rabbit by artificial stimulation and the use of a vaginal sponge or a condom is not practical (*27*), so the seminal fluid was recovered by allowing a buck to serve a female normally and then recovering as much fluid as could be obtained from vagina and uterus by means of a catheter and rubber bulb. This method seems to be the only practical one for an experiment of this kind on rabbits, and moreover it is simple and easily operated and the amount of fluid which can be recovered is large; but it has the disadvantage that any secretions or cellular residues or bacteria within the vagina are also drawn into the catheter and any effect which they may produce upon the spermatozoa can not be separated out. Moreover, an infection is readily spread from female to female by this method, since the fluid can not be sterilized without killing the sperma-

tozoa. Also it is impossible to be certain that all the spermatozoa ejaculated by the buck have been removed and therefore the female from which the seminal fluid has been recovered can not be used for an artificial insemination with a centrifuged portion of that fluid. In fact, the records show that in this experiment the females from which as much fluid as possible was recovered were more likely to become pregnant than were those which were inseminated artificially. This furnished an excellent control for the insemination experiments, but it made necessary the use of a much larger number of females. An attempt was made to avoid this difficulty by using for recovering the semen females upon which the operation of salpingectomy had been performed. Since they could not become pregnant, they could be used very frequently and a small number of them would suffice for recovering enough seminal fluid to inseminate artificially a very large number of normal females. However, in practice this did not work out so well because these females did not come in heat any oftener than normal females, that is at intervals of 12 to 15 days, and a large number of them would have been necessary if one were to have been available every time a normal female was to be inseminated. Moreover, after several months the females upon which salpingectomy had been performed became very fat and almost entirely ceased to come in heat, and, since a number of the normal females would accept service even when they were pregnant, the use of operated females was discontinued. Only three such females were used in this experiment, and the conclusions derived from their behavior are of little value by themselves, since individuality is such a large factor in determining behavior, but the conclusions reached agree very well with those reached by Lloyd-Jones and Hays (*27*) from more extensive observations.

Ordinarily all the females which had not been bred within the preceding 10 days were tested each morning or every alternate morning to find out whether they were in heat. When two or more were found in heat the same morning, a buck was allowed to serve one of them naturally as many times as he would in about 15 minutes, which was usually twice, but sometimes three times or more, and more rarely only once. Then the female was removed and all the fluid that could be recovered from her vagina and uterus by two or three successive insertions of the catheter was obtained. If the amount of fluid was large enough to be handled easily, the female was returned to her hutch and was not tested again for 10 days, but if the amount was too small the catheter was filled with warm Ringer's solution which was injected into the uterus and allowed to remain 2 or 3 minutes. Then the catheter was again thrust in and all the liquid possible was withdrawn. In this way more semen was recovered, diluted to a somewhat uncertain extent, so that it could be handled and centrifuged very conveniently.

The catheters [5] used were made from pieces of glass tubing with an outside diameter of 5 to 6 mm., with a slight bend near one end and a bulbar expansion near the other. The total length was 18 cm., and the part which could be thrust into the vagina and uterus of the female was about 12 to 12.5 cm. long. The uterus of the rabbit does not have a very definite cervix or os, and the body of the uterus is short and small while the horns are relatively long. Because of this, the catheter was thrust not only into the vagina but also into the uterus and usually even a considerable distance into one of the horns of the uterus when withdrawing liquid.

CHARACTER AND TREATMENT OF THE FLUID

The fluid recovered consisted of a combination of semen and vesicular and prostatic secretions from the male and the vaginal and uterine secretions from the female. It was usually opaque and of a pale milky color; sometimes it was of a dense yellowish or milky white color, and sometimes it was almost transparent. If recovered from a female within two or three days after parturition it usually had a reddish or brownish tinge and evidently contained some blood. Sometimes urine would be contained in the liquid. This would usually give it a rather transparent yellow color and the spermatozoa would be found to be nearly or quite motionless. Often there were very many round motionless cells about the structure of which very little could be made out. These were often associated with cases of known pus infection in the uterus of the female from which the fluid was drawn, but whether this relation was significant

[5] This catheter or pipette, first devised in this form by Bachhuber, has been used and figured by Guyer in his "Studies on Cytolysins" (*13, p. 208*).

is not known. Often there were transparent gelatinous masses as much as 2 or 3 mm. in diameter. No attempt was made at a chemical analysis of the liquid or to determine its physical properties except such as were visible. The liquid was examined microscopically as soon as recovered. If no active spermatozoa were found, the liquid was discarded, but if there was a fair number of active spermatozoa, the liquid was used.

Sometimes the liquid was centrifuged untreated, but usually in order to get a convenient volume it was diluted to some degree with Ringer's solution. The only noticeable effect of dilution was that it resulted in a more distinct separation of the spermatozoa and cells from the liquid upon centrifuging and fewer of them were found in the inside of the centrifuge tube. Presumably, therefore, it decreased the specific gravity or the viscosity of the fluid.

The centrifuging was done in the earlier experiments with a Bausch & Lomb hand centrifuge with which it was possible to govern the centrifugal force by regulating the speed at which the operator turned the handle. The speed ordinarily used was about 1,600 r. p. m. The later experiments were carried out with a Bausch & Lomb electric centrifuge which had a lowest speed of about 1,600 r. p. m. and a second speed of about 2,000 r. p. m. The liquid was placed in the centrifuge in glass tubes 12.5 cm. long with an inside diameter of about 6.5 mm., and therefore a capacity of about 4.15 c. c. They were so arranged that when revolving the extreme bottoms of the tubes were about 30 cm. apart and the tops were about 5 cm. apart. Therefore, the amount of centrifugal force applied varied in the different regions of the tube. The centrifuge tube containing the liquid was kept in a water bath at a temperature of 35° to 37° C. until it was placed in the centrifuge which was at room temperature. This naturally varied somewhat at different seasons. After the centrifuging, the tubes were again placed in the warm-water bath before injection. The length of time during which the tubes were centrifuged varied from 2 to 10 minutes. When prolonged much beyond 10 minutes, the spermatozoa were so nearly all thrown to the bottom of the tube that it seemed useless to try insemination with the liquid from the top.

INSEMINATION

The liquid was injected into the uterus of the females with another catheter which was so long that nearly all the liquid must have been deposited in the upper end of the uterus or in one of the horns. Some females were inseminated when not in heat and others showed unmistakable signs of heat but had not actually copulated with the test males. This was done to test whether copulation is necessary to ovulation in the rabbit as maintained by Marshall (*29*). Some were inseminated with the untreated liquid just as it was recovered. Others were inseminated with liquid which, aside from dilution, had received no treatment except that it had been allowed to remain in test tubes at low temperature for periods of from 1 to 48 hours. However, the majority were inseminated with centrifuged material and these were grouped in two classes according to the part of the centrifuge tube from which the material was taken. The top one-third of the liquid in the tube, that which had been on the inside while the centrifuge was running, was carefully drawn off with the catheter, and females inseminated with this portion were recorded as "inside insemination." The middle one-third of the fluid was discarded entirely except that occasionally microscopic slides were made from it. The bottom or outer one-third, which also contained the sediment, was stirred up and injected into a single female. Such inseminations were recorded as "outside inseminations."

ISOLATION OF FEMALES

Females used for breeding were kept in individual hutches from which they were removed only for testing whether they were in heat. All females when bred in any manner were left in their hutches for 10 days before being again tested.

CHECKING THE SEX OF THE OFFSPRING

The sex of the living rabbit can not often be identified before it is 3 weeks old and sometimes not until it is 8 weeks old. To make possible the production of a larger number of offspring from a given number of females, the young were often killed immediately after birth and the mother bred again, as that is one of the surest times to

induce pregnancy. The young killed in this way, together with those which died in the first few weeks, make up about half of the total number produced, and their sex was identified solely by autopsy. All young or partially grown rabbits which died were also autopsied to verify the records of their sex as determined from external examination. The ovary and testis have very distinctive shapes in the young rabbits even when just born. This is due largely to the distinct firm union of the epididymis to the testis and the small bulb where the epididymis curves at the anterior end of the testis as contrasted to the loose attachment of the Fallopian tube to the ovary. The positions of the ovary and testis are not so distinctive, for the testis is usually just starting to descend when the rabbit is born; but it descends in a very few days and, moreover, the *gubernaculum testis* is so prominent even at birth that it usually would serve for identification. Taken in connection with the shape and position of the testis, there is no room for doubt as to the sex of a young rabbit unless it has been partly eaten by the mother or has been dead long enough to have started to decompose. The female rabbit quite frequently eats her newborn young, and these, together with others about which the records are not quite clear, constitute the group of "unknown sex" in these records.

MICROSCOPIC WORK

To parallel the breeding work with the rabbits and serve as a control by determining the effect of centrifuging upon the measurements of spermatozoa, smears were made of the untreated fluid just as it was recovered from the uterus or after dilution. Again after centrifuging there were smears made both of the "inside" and "outside" material and sometimes of material from intermediate positions. The material was placed upon a clean slide or upon one which had been thinly coated with fresh albumen and was either heated first to fasten it to the slide and then was fixed in Bouin's fluid or was fixed in Bouin's fluid direct without any heating. As good results were secured without as with the heating, except that when the material had been much diluted there was sometimes not enough of its own protein to fasten it to the slide without heating and it washed off while being fixed in the Bouin's fluid. The smears were stained in Heidenhain's iron haematoxylin or in Delafield's haematoxylin, with eosin as a counter stain.

The head lengths of a large number of spermatozoa were measured on a number of these slides, but since this work necessarily requires a great deal of time only a small fraction of the whole number of slides made could be measured. The first measurements were made with a Spencer micrometer eyepiece, but all the later measurements were of drawings made with a camera lucida, as this made it unnecessary to touch the microscope tube at all during measurement and thus removed a possible slight error caused by jarring the tube when touching the micrometer screw. However, when the accuracy of both methods was tested by measuring 100 spermatozoa twice the micrometer method was found to be slightly more accurate. The coefficient of correlation between first and second measurements was $+0.671 \pm 0.037$ for the camera lucida method and $+0.761 \pm 0.028$ when the ocular micrometer was used. Seven hundred spermatozoa were measured on each of four normal slides, two containing the outside centrifuged and one the inside centrifuged liquid. The "inside" slide, one of the "outside" ones, and one of the normal ones were all made from the same sample of recovered liquid.

Microscopic work was also done on swine spermatoza to determine the effect of centrifuging. Smears were made, in the same way as described for the rabbit, of the normal material and also of the material as taken from different positions in the centrifuge tube immediately after centrifuging. Measurements were made entirely by the camera lucida method of 700 spermatozoa on each of three normal slides, three "inside" slides and one "outside" slide.

VITALITY OF SPERMATOZOA

A number of observations were made upon the effect of heat and cold upon the length of time spermatozoa remained motile. A bull was killed and the testicle was immediately removed and placed in a refrigerator and examined daily afterward by making a fresh cut in the epididymis and looking for motile spermatozoa in the liquid which oozed out. A few motile spermatozoa were still found on the eighth day, but through an oversight all the ice was allowed to melt away that afternoon and on the ninth day the odor of putrefaction was quite marked and no motile spermatozoa could be found. In the course of the work on the swine and rabbit spermatozoa the liquids were often kept in a test tube at temperatures varying

from 0° to 20° C. until all motility disappeared or until an odor of putrefaction was noticeable. In the case of the lower temperature some motility was observed as long as 60 hours after ejaculation in some samples, and almost always as long as 48 hours. With temperature as high as 18° to 20° all motility disappeared sooner, sometimes in less than 24 hours.

Temperature was not recorded except at the time of observation, and temperature control was not accurate, so that the only conclusion which can be drawn is the general one that spermatozoa when kept in a liquid in vitro retain motility much longer at lower than at higher temperatures, and therefore that the checking of their activity is due to bacterial growth in the medium or the accumulation of the by-products of their own activity or the exhaustion of their own supply of energy due to this greater activity, rather than to the mere lapse of time. Possibly they would remain active much longer inside the uterus where the toxic products of their own or of bacterial activity would diffuse away to a considerable extent. Not all the spermatozoa in the same sample remained active for the same length of time, but usually a great many were inactive at the end of 24 hours, even though some remained active for more than 48 hours. Whether motility is a sufficient index of fertilizing ability is not revealed by these data. There is only the fact that two litters were produced after insemination with liquid which had been kept for 24 hours, in the one case at 10° to 18° C. and in the other at 13° to 17°.

DISCUSSION OF RESULTS

SEX OF ALL RABBITS PRODUCED

Table I presents the sex of all the rabbits which have been produced in the flock of the genetics department of the University of Wisconsin in the last 10 years. The classes marked "alcoholized" or "lead" are from former experiments (*6*, *7*), the plan of which was to mate albino females to two males on the same day, one of the males being normal and the other in a chronic state of lead or alcohol poisoning. One of the males was an albino and the other was homozygous for the pigment-producing factor and therefore the paternity of the young produced could be known. The individuals in the class marked, "dam had not copulated" are counted again in their appropriate insemination class, but are only counted once in the grand total. Also the individuals of the "fluid washed out" class are counted again in the "fluid withdrawn" class, but are only counted once in the grand total. The next to the last column in Table I gives the probable error to be expected on the basis of exact equality in the numbers of each sex. The last column in Table I shows for each method of breeding just what proportion the deviation from exact equality bears to its probable error and therefore whether these deviations are likely to be the result of anything but chance.

In this and the following tables the probable errors and the deviations are calculated upon the basis of an expectation of exact equality in the sex ratios. As far as the chromosome theory of sex goes, equality is to be expected, but, as

TABLE I.—*Sex of rabbits produced in flock of the genetics department, University of Wisconsin, during 10 years*

Type of litter		Sex			Probable error 1:1	Deviation—probable error
		Males	Females	Unknown		
Sire alcoholized		6	12	7	1.43	2.10
Lead	Sired by poisoned buck	102	93	20	4.71	.96
	Sired by normal buck	74	63	[a] 32	3.95	1.39
Artificial insemination	Dam had not copulated	12	10	3	1.6	.63
	Fluid untreated	11	17	7	1.8	1.68
	Fluid cooled	18	23	6	2.16	1.11
	Centrifuged, "inside"	64	64	3	3.82	
	Centrifuged, "outside"	103	97	14	4.77	.63
Natural service	Fluid withdrawn	170	205	28	6.53	2.68
	Fluid washed out	18	27	1	2.26	1.99
	No treatment	421	394	174	9.63	1.40
Grand total		969	968	291	14.84	.03

[a] Some of these may belong in the row above.

already mentioned, observation has apparently shown that there are slight but consistent deviations which vary for different species (*33*). Among most mammals it seems that there is a slight excess of males, the normal sex ratio being usually somewhere between 103 and 110 males per 100 females. This may very easily be due to some difference in the motility or viability of the two classes of spermatozoa. The presumption is that in the rabbit also there will normally be a slight excess of males, and the data from this flock for the litters produced by a natural service without any treatment afterward support that assumption, for there were 421 males and 394 females, a ratio of 106.9 ♂ ♂ : 100 ♀ ♀.

However, no report of large numbers has been found for the rabbit. The numbers available are too small for calculations to be based upon ratios drawn from them for a standard, and therefore the calculations in these tables are based upon exact equality as a rigid standard to be given up only when enough data are available to establish definitely how far the normal sex ratio in the rabbit does deviate from equality. The probability that the normal sex ratio is somewhere between 105 and 108 males per 100 females should be kept in mind, however, in considering the results given in these tables.

The common method of stating sex ratios in terms of the number of males per 100 females is open to criticism in that a change in the number of males produced is not given equal numerical value with an equal change in the number of females produced. Thus in a population where the sexes are equal, if a change were made which reduced by one-fourth the number of males born the sex ratio would be given at 75 ♂ ♂ : 100 ♀ ♀, whereas in the same population if the change had reduced the number of females produced by one-fourth the sex ratio would have been stated as 133⅓ ♂ ♂ : 100 ♀ ♀. In the one case the ratio would be described as 25 points below the normal and in the other as 33⅓ points above the normal, although the magnitude of the changes had been the same fundamentally, but took place in different sexes in the two cases. This seems to be a minor point, but it tends to obscure what may be a very vital relation. For example, in her work on selection for abnormal sex ratios in the rat, King (*23*) states that in the high male line after the seventh generation the sex ratio was 122.3 ♂ ♂ : 100 ♀ ♀, while in the low male line for the same period it was 81.8 ♂ ♂ : 100 ♀ ♀. Now it is not readily apparent without calculation that a ratio of 81.8 ♂ ♂ : 100 ♀ ♀ is the same as a ratio of 100 ♂ ♂ : 122.25 ♀ ♀, or almost exactly the same as the ratio in the high male line except that the sexes are reversed. If these ratios had been expressed in percentages, that fact would have been apparent at once and, taken in connection with the fact that selection appeared on the whole to have very little effect after the twelfth generation, would have brought home a conclusion not emphasized in that paper—namely, that in the albino rat selection can alter the sex ratio in either direction with equal ease but can only carry it the same distance on either side of equality. That fits in very well with the hypothesis that the results which she secured were due to sex-linked lethal factors or factors whose lethal action was confined to one sex, and suggests that the ratio which she adopts as a normal, 105 ♂ ♂ : 100 ♀ ♀, is really the result of some such factor as a difference in motility of the two types of spermatozoa. There seems to be no good reason for expressing sex ratios in terms of the number of males per 100 females except that it is customary and magnifies the differences between ratios.

The attempt to develop a practical method of sex control by means used in these experiments was not successful. In Table I, where the different methods of breeding are compared, the only sex ratio which deviates far enough from equality and concerns numbers enough to approach statistical significance is that of the young produced by a natural service where all the fluid possible was withdrawn with a catheter. If the normal sex ratio for the rabbit be taken as 106.9 ♂ ♂ : 100 ♀ ♀, as it was found to be with the litters produced in the normal way in this flock, then the deviation of this class from expectation becomes more than 3.64 times the probable error, and, looking at it from a purely statistical standpoint, it may be said that the deviation is probably significant. However, when it is asked what factor has changed the sex ratio and how it has operated to produce this result, the answer is not apparent. The only readily apparent change which has been made in this type of breeding is a reduction in the number of spermatozoa which have been left in the uterus and vagina, but just how this could influence the sex ratio is not apparent. A reduction in the number of spermatozoa present might cause an already abnormal sex ratio to approach nearer equality in a way analogous to the differential

growth rates of pollen tubes as shown by Correns (*8*), but that a reduction should cause the sex ratio to deviate much more on the other side of equality is hardly explainable upon that basis.

One other effect which the withdrawal of the seminal fluid with a catheter might have had is to carry parts of the fluid much farther up into the uterus than a natural service would have done, but even if this were done to a considerable extent there does not seem to be any reason to think that more of the female-producing than male-producing spermatozoa would be carried up into the uterus nearer to the unfertilized ova. Furthermore, if it were only a matter of pushing the spermatozoa far up into the uterus, all the litters produced by artificial insemination should show a very high proportion of females, whereas actually there was only a bare majority of females. Since there is apparently no physical basis for this abnormal sex ratio, judgment must be reserved as to its real significance until there are more data available. However, it is certain that none of the other methods of breeding has produced a significant change in the sex ratio, for if, instead of equality the ratio of 106.9 males be taken as normal, the deviations from expectation become in general even less than they are when based upon equality.

SEX OF OFFSPRING IN RELATION TO LENGTH OF GESTATION PERIOD

That there is no relation between the sex of the offspring and the length of the gestation period is shown by Table II. Here the nearest whole number of days between the hour of breeding and the hour of finding the litter is given. Since many of the litters may have been as much as 12 hours old when they were found, it is apparent that while some of them may belong in a period one day shorter than that in which they are placed, none of them belong in a longer period.

That the length of the gestation period would vary with the sex ratio of the litter is hardly to be expected, since nearly every litter contains both males and females, and even if the males require a longer or a shorter time to complete their development still the litter must all be born at the same time and only the exceptional cases where the litter is all of the same sex might be expected to show this relation. Since the extreme gestation periods as shown in Table II do not give extreme ratios and since the average gestation period of the female offspring was less than 0.02 days in excess of that of the males, it may be concluded that in the rabbit the sex of the offspring bears no relation to the length of the gestation period. There may, however, be some such relation in uniparous animals where an embryo of one sex during its entire course of development is free from possible influences from embryos of the other sex in the uterus beside it, and it is a fairly common belief among breeders of horses and cattle that there is such a relation in those animals.

TABLE II.—*Sex of offspring and length of gestation period*

Length of gestation period	Sex of offspring			Deviation—probable error
	Males	Females	Unknown	
Days				
Unknown	179	144	71	2.89
26	1	3		
28	10	6	1	
29	15	13	1	
30	114	134	44	1.88
31	175	198	29	1.77
32	104	101	13	.31
33	16	17	2	
34	11	8	2	
35	2	4	1	
39		3		

SEX RATIOS OF PROGENIES OF INDIVIDUAL MALES AND FEMALES

That such deviations from a normal sex ratio as may exist are not due to the progeny of a single male which happen to be grouped together is shown by Table III, which gives the sexes of the progeny of all males which have as many as 50 progeny whose sex is known.

TABLE III.—*Sex of progeny of males*

Sire	Sex of offspring			Deviation—probable error
	Males	Females	Unknown	
274.3	32	30	18	0.38
301.3	45	50	6	.76
316.1	31	47	29	2.68
411.1	53	46	11	1.04
397.1	60	70	8	1.30
440.1	39	36	3	.51
391.1	37	46	7	1.46
515.4	22	28	8	1.26
534.1	47	38	8	1.48
20.2	28	23	7	1.04
20.2 and 21.4 (Lead experiments)	73	70	3	.37
26.8 and 20.2 (Lead experiments)	69	69	38	

Table IV presents the corresponding facts for all females which have 25 or more offspring of known sex.

TABLE IV.—*Sex of progeny females*

Dam	Sex of offspring			Deviation—probable error
	Males	Females	Unknown	
5.1	14	17	4	0.8
12.2	30	16	5	3.06
21.2	17	21	12	.96
26.1	21	10	2	2.93
26.2	22	21	14	.22
26.4	11	15	4	1.16
54.1	14	14		
56.3	16	22	1	1.44
65.5	17	16	4	.26
65.6	17	13	8	1.08
68.5	12	18	3	1.62
293A2	13	12	1	.30
297.1	15	13	12	.56
369.2	14	16	5	.54
370.2	14	16	1	.54

If the observed irregularities in the sex ratio were due to the presence of sex-linked lethal factors in some of the breeding stock, the records of individual sires and dams should show this fact. Tables III and IV do show a number of irregularities, but perhaps no more than might be expected upon the basis of chance deviation. The breeding records of ♂316.1, ♀12.2 and ♀26.1 deviate just far enough from expectation that it is possible but not necessary to regard them as significant. All three animals are now dead and it is impossible to apply breeding tests to settle the question.

SEX IN RELATION TO DILUTION OF SEMINAL FLUID

The degree of dilution of the recovered liquid has an effect upon completeness of separation of the liquid and the solids contained in it, as already mentioned, but that it does not seem to have had an effect upon the sex ratio of the offspring produced is shown by Table V. The figures given for degree of dilution are in terms of the volume of Ringer's solution, which was added to the liquid recovered. Three volumes was the amount usually added, but for various reasons, usually to secure enough volume to be handled easily, other dilutions were sometimes made.

RELATION OF SPEED AND DURATION OF CENTRIFUGING TO SEX OF OFFSPRING

That the speed at which the centrifuging was done did not affect the sex of the offspring produced is shown by Table VI. The true speed of the electric centrifuge was undoubtedly somewhat greater than the 1,600 r. p. m. and the 2,000 r. p. m. measured, because the cover was on during the actual centrifuging, and therefore the air resistance was less than when the speed was being measured with a revolution counter.

TABLE V.—*Degree of dilution and sex of offspring*

Degree of dilution	Sex of offspring			Deviation—probable error
	Male	Female	Unknown	
None	32	28	1	0.77
1 to 2	21	27	5	1.28
3	79	92	8	1.47
4 to 5	16	12		1.12
8 to 12	13	4	1	3.23
Uncertain	22	11	4	2.84

TABLE VI.—*Speed of centrifuging and sex of offspring*

Speed	Sex of offspring			Deviation—probable error
	Male	Female	Unknown	
1,600 r. p. m. (hand centrifuge)	61	72	3	1.41
1,600 r. p. m. (electric centrifuge)	97	85	3	1.32
2,000 r. p. m. (electric centrifuge)	10	5		1.91
Odd and unknown speeds	4	6	1	.94

That the length of time the liquid was centrifuged is not a controlling factor is shown by Table VII, which gives the sexes of the rabbits produced from spermatozoa which had been centrifuged for varying lengths of time.

TABLE VII.—*Time of centrifuging and sex of offspring*

Time centrifuges	Sex of offspring			Deviation—probable error
	Male	Female	Unknown	
0 to 2 minutes	22	21		0.23
2 to 4 minutes	31	41	1	1.75
4 to 6 minutes	30	33	10	.56
6 to 10 minutes	75	62	6	1.65
10 to 15 minutes	7	5	6	.85
Unknown		4		2.97

MISCELLANEOUS FACTORS

The number of copulations as related to the sex of the offspring showed no effect, but the females were in excess in all classes except the four-copulation and five-copulation classes, in each of which the total number involved is less than 15. A large effect could scarcely be expected (*16*) because the number of copulations was in no case greater than five, and where there was more than one the liquid from the preceding copulations was mixed all together with that of the last, and therefore it is impossible to know whether a given one of the young was produced by a spermatozoon from the first or a later copulation.

It is hardly to be expected that the amounts of liquid recovered by the methods used in this experiment would be comparable enough to the amounts actually ejaculated to show a difference in the sex ratios produced by different amounts, even if such a difference existed. The sexes were either exactly even or there was a slight excess of females, which approached significance in only one case and did not seem to vary uniformly as related to the amount of liquid.

The microscopically distinguishable differences in the activity of the spermatozoa did not show any corresponding differences in the sex ratios of the offspring produced. This is not surprising, since no liquid was used for insemination unless there was at least a moderate number of fairly active spermatozoa to be seen in it.

RELATION OF TYPE OF BREEDING TO SUCCESS IN INDUCING PREGNANCY

The records kept before the centrifuging was begun were not detailed enough to permit taking any figures as to services which were natural in every way. Table VIII gives the number and percentage of services and inseminations which resulted in pregnancy and also the number which did not result in pregnancy. The percentage of success is figured only for those methods which were tried at least 30 times.

Several factors combine to make the data in Table VIII only approximate. A number of females would accept service while pregnant and a number were barren either through an infection in the Fallopian tubes or for some other reason. Such females were kept when there was room for them and were used for the recovery of seminal fluid, because the main object of the experiment was the production of the largest possible number of young by insemination with centrifuged or with cooled liquid, and so it was desired to use the fertile females for insemination. Therefore the true percentage of success for the natural-service type of breeding where the liquid was withdrawn is considerably higher than the figure given. The very low percentage of success where the fluid was cooled for some time before insemination brings down the percentage of success in the total inseminated, and in the inseminated but not centrifuged much lower than it should be in comparison to the

TABLE VIII.—*Relation of type of breeding to success in inducing pregnancy*

Type of breeding				Failed	Succeeded	Per cent of success
Natural service	Fluid withdrawn without washing			300	69	18.7
	Fluid washed out			10	7	
Inseminated	Normal	Undiluted		31	4	11.4
		Diluted		6	0	
		Delayed		1	1	
	Centrifuged	Undiluted	Whole sample	4	0	
			Inside sample	30	4	11.8
			Outside sample	23	9	28.1
		Diluted	Whole sample	1	0	
			Inside sample	172	35	16.9
			Outside sample	157	36	18.7
	Held some time at abnormal temperatures.	Cold	Undiluted	79	3	3.7
			Diluted	55	5	8.3
		Warm	Undiluted	23	1	
			Diluted	6	0	
Total undiluted				190	21	10.0
Total diluted				397	76	16.1
Total inseminated				588	98	14.3
Total natural services with semen removed				310	76	19.7
Total centrifuged				387	84	17.8
Total inseminated but not centrifuged				201	14	6.5

natural service with semen removed or to the total centrifuged—that is, the apparent conclusion that centrifuging increases the chances of pregnancy over insemination without centrifuging is probably due only to the harmful treatment given the uncentrifuged fluid. Just why dilution of the liquid increased the chances of pregnancy is not known; the Ringer's solution may have stimulated the spermatozoa to greater activity or in some other way may have made fertilization more easy. Probably the absolute percentage of success should be higher in nearly every case, because some barren females were used which were not known to be barren and some females died or were sold while still pregnant.

The general conclusion can be drawn that spermatozoa retain their activity and fertilizing ability under more adverse conditions than is popularly supposed. Being kept in an ice box for eight days did not completely destroy their motility. Neither did the unusual handling involved in centrifuging and artificial insemination destroy their motility. Whether motility and fertilizing ability are the same thing is another question; but even though they are not, many young are produced from centrifuged spermatozoa and many were produced from spermatozoa exposed to low temperature for periods up to 24 hours. The only factor found to injure seriously the activity of the spermatozoa was the presence of urine in the liquid, and no experiments were made to determine whether this toxicity was due to acidity or to a toxic action of some other substance in the urine.

MONTHLY DISTRIBUTION OF LITTERS BORN IN LAST 10 YEARS

The breeding season of the rabbit under natural conditions is usually given as in the late winter or early spring. Table IX gives the number of litters by months which were born in the last 10 years in this flock.

These figures can be regarded only as giving an approximation to the true breeding season because so many other factors varied. Thus the breeding flock was much larger at some times than at others. Also the breeding work was pushed more vigorously some months than others, and during at least 3 years no breeding was done during July and August. The figures are presented as tending to support the idea that the breeding season is in the spring and late winter but denying that breeding is confined to that season.

TABLE IX.—*Monthly distribution of litters born in last 10 years*

Month	Number of litters	Month	Number of litters
January	28	July	29
February	44	August	21
March	75	September	14
April	66	October	15
May	89	November	21
June	49	December	40

OVULATION

As quoted by Marshall (*29*), Heape (*17*) maintains that ovulation in the rabbit is dependent upon the stimulus of coition and can not be induced by artificial stimulation, but Weil (*41*) maintains that ovulation may take place without coition just after the delivery of a litter, and Iwanoff (*19, 20*) induced pregnancy by artificial insemination of rabbits just after parturition. That neither of those views goes far enough is shown by the four litters produced in these experiments by artificial insemination of females which had neither delivered a litter nor copulated within less than 13 days previous and were not even placed with a male for 10 days following. A fifth litter was produced by a female which had been inseminated the day after she had produced a litter. She was evidently in heat but was not allowed to copulate. This is a parallel case to those of Iwanoff. It is certain that ovulation can take place without the stimulus of coition, but it is not yet certain that ovulation proceeds without any psychic stimulus from the courtship of the male as it does in the case of the cow. It may be that the stimulus of courtship is necessary, as it is in pigeons, even though it may be two females which court each other. In the case of these four litters the females were placed with the test males and showed signs of heat, but were removed before they could copulate; hence the physic stimulus of courtship was present; but that it was necessary seems improbable, since the females often show signs of heat immediately upon being placed with other females, mounting them and sometimes even having an orgasm when mounted, although no male is near and the female in heat has been alone in her hutch for 24 hours.

MICROSCOPIC WORK

The measurements of rabbit spermatozoa from seven different slides are shown in Figures 1 to 7, together with the theoretical normal curves for populations of those sizes with those means and standard deviations. Slides Nos. 370, 371, and 372 were all made from the same sample of recovered semen. Slide 277 was made from semen of the same buck but recovered at a different time.

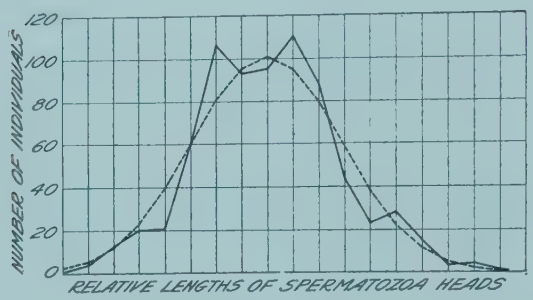

FIG. 1.—Actual (solid line) and theoretical (broken line) frequency distribution of the head lengths of untreated rabbit spermatozoa. The actual distribution is clearly bimodal and the probability that it is only a chance deviation from the theoretical distribution is less than 0.001. (Slide No. 370)

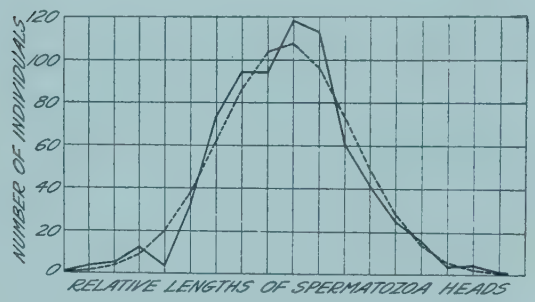

FIG. 2.—Actual (solid line) and theoretical (broken line) frequency distribution of the head lengths of rabbit spermatozoa taken from the inside of the centrifuge tube after being centrifuged 5 minutes at 1,600 r. p. m. The liquid had first been diluted with three times its volume of Ringer's solution. Made from the same sample of semen as that in Figures 1 and 3. The probability that the actual distribution is only a chance deviation from the theoretical normal curve is 0.207. (Slide No. 371)

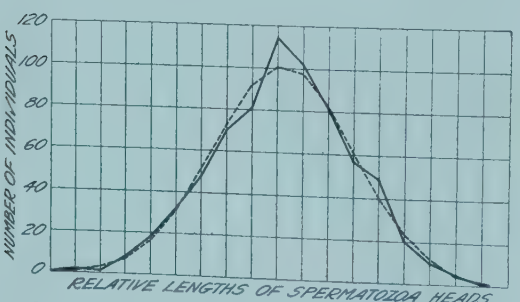

FIG. 3.—Actual (solid line) and theoretical (broken line) frequency distributions of the head lengths of rabbit spermatozoa taken from the outside of the centrifuge tube after being centrifuged five minutes at 1,600 r. p. m. The liquid had first been diluted with three times its volume of Ringer's solution. Made from the same sample of semen as that used in Figures 1 and 2. The probability that the actual distribution is only a chance deviation from the theoretical normal curve is 0.84. (Slide No. 372)

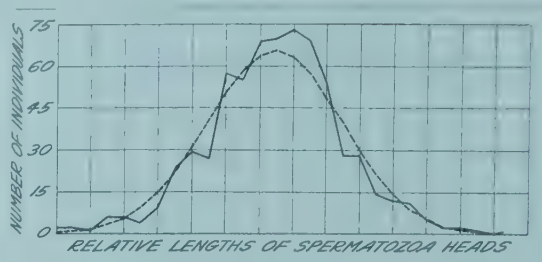

FIG. 4.—Actual (solid line) and theoretical (broken line) frequency distributions of the head lengths of untreated rabbit spermatozoa. The probability that the actual distribution is only a chance deviation from the theoretical is 0.12. (Slide No. 271)

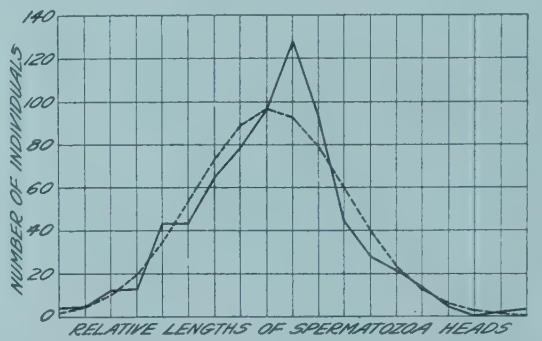

FIG. 5.—Actual (solid line) and theoretical (broken line) frequency distributions of the head lengths of untreated rabbit spermatozoa. The probability that the actual distribution is only a chance deviation from the theoretical is less than 0.001. (Slide No. 277)

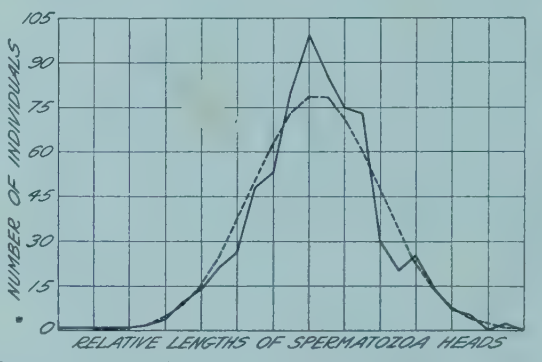

FIG. 6.—Actual (solid line) and theoretical (broken line) frequency distributions of the head lengths of untreated rabbit spermatozoa. The probability that the actual distribution is only a chance deviation from the theoretical is 0.029. (Slide No. 249)

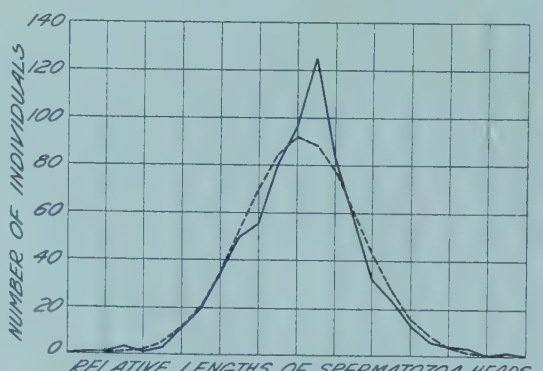

FIG. 7.—Actual (solid line) and theoretical (broken line) frequency distributions of the head lengths of centrifuged rabbit spermatozoa. From the outside portion of the centrifuge tube after being centrifuged eight minutes at 1,600 r. p. m. The probability that the actual distribution is only a chance deviation from the theoretical is 0.09. (Slide No. 263)

The measurements of swine spermatozoa from seven different slides are shown in Figures 8 to 14, together with the theoretical normal curves [6] for populations of those sizes with those means and standard deviations. Slides Nos. 519, 531, and 536 were made of semen out of the testicle of one boar, slides Nos. 522 and 526 were made from the fluid recovered from a natural service by the younger boar, and slide 537A was made of semen from the testicle of still a third boar. Two separate sets of measurements were made on slide 531 (figs. 10 and 11).

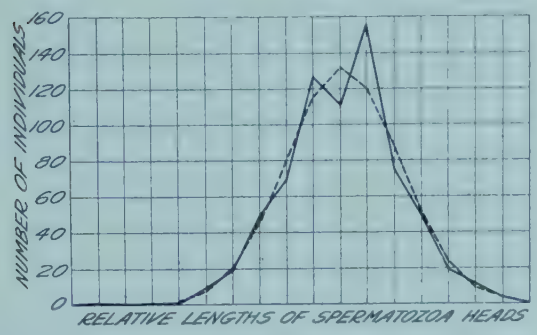

FIG. 8.—Actual (solid line) and theoretical (broken line) frequency distributions of the head lengths of untreated swine spermatozoa taken from the testicle of an old boar castrated three or four hours before this slide was prepared. The probability that the actual distribution is only a chance deviation from the theoretical is 0.017. (Slide No. 519)

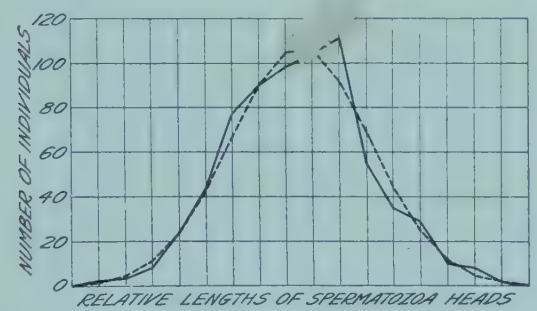

FIG. 9.—Actual (solid line) and theoretical (broken line) frequency distributions of the head lengths of centrifuged swine spermatozoa. From the extreme outside end of the centrifuge tube after five minutes of centrifuging at 1,600 r. p. m. From the same sample of semen used in preparing Figures 8, 10, and 11. The probability that the actual distribution is only a chance deviation from the theoretical is 0.31. (Slide No. 536)

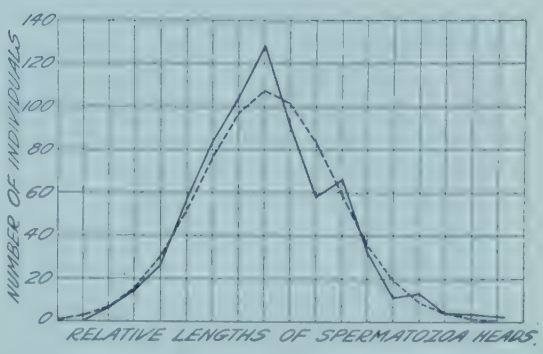

FIG. 10.—Actual (solid line) and theoretical (broken line) frequency distributions of the head lengths of centrifuged swine spermatozoa. From the extreme inside end of the centrifuge tube after being centrifuged five minutes at 1,600 r. p. m. From the same sample of semen used in preparing Figures 8 and 9 and from the same slide from which Figure 11 was prepared. The probability that the actual distribution is only a chance deviation from the theoretical distribution is 0.041. (Slide No. 531)

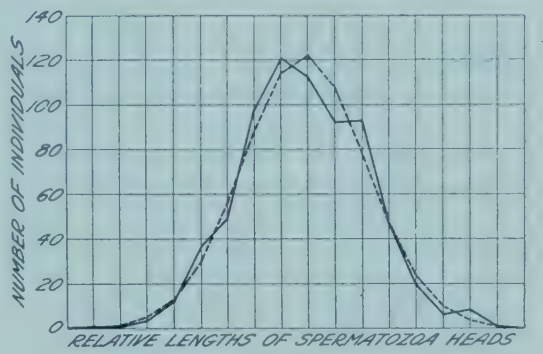

FIG. 11.—Actual (solid line) and theoretical (broken line) frequency distributions of the head lengths of centrifuged swine spermatozoa. From the extreme inside end of the centrifuge tube after five minutes of centrifuging at 1,600 r. p. m. From the same sample of semen used in preparing Figures 8 and 9 and from the same slide from which Figure 10 was prepared. Figures 10 and 11 were made from measurements made on spermatozoa from different parts of the same slide and thus serve as a control upon the accuracy of the measurements. The probability that the actual distribution is only a chance deviation from the theoretical distribution is 0.30. (Slide No. 531)

[6] The theoretical normal curves were constructed according to the method explained in West's "Introduction to Mathematical Statistics" (*42*). The curve is completely determined by three things—namely, its mean, standard deviation, and the number of individuals involved. These three figures for each theoretical curve are taken from its corresponding actual curve. The probability that the actual curve is only a chance deviation from the theoretical normal one is obtained by the X^2 method of testing the goodness of fit of one set of observed data to a corresponding set of expected data (*15*).

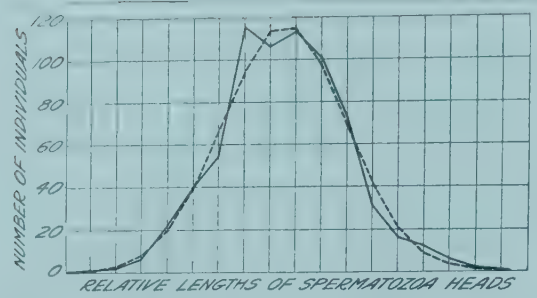

FIG. 12.—Actual (solid line) and theoretical (broken line) frequency distributions of the head lengths of untreated swine spermatozoa. Recovered from the vagina of a sow after natural service. The probability that the actual distribution is only a chance deviation from the theoretical is 0.26. This is from the same sample of semen as was used in the preparation of Figure 13. (Slide No. 522)

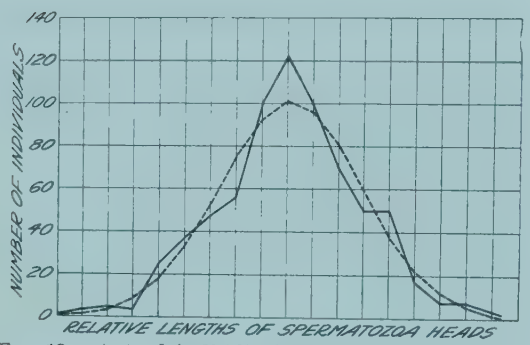

FIG. 13.—Actual (solid line) and theoretical (broken line) frequency distributions of centrifuged swine spermatozoa. From near the inside end of the centrifuge tube after five minutes of centrifuging at 1,600 r. p. m. From the same original sample of semen from which Figure 12 was made. The probability that the actual distribution was only a chance deviation from the theoretical distribution is 0.008. (Slide No. 526)

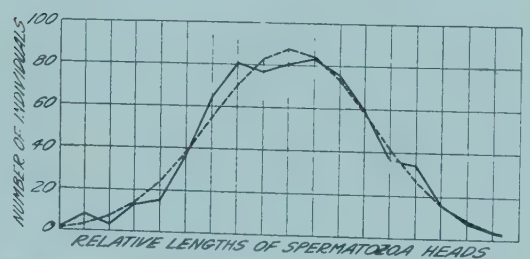

FIG. 14.—Actual (solid line) and theoretical (broken line) frequency distributions of untreated swine spermatozoa. The spermatozoa were obtained from the testicle of an aged Poland-China boar immediately after castration, and the milky fluid which was recovered from the epididymis was diluted with about six volumes of Ringer's solution and slide 537A was made directly from this. The probability that the actual distribution is only a chance deviation from the theroetical distribution is 0.55. (Slide No. 537A)

The microscopic work on the rabbit spermatozoa was directed toward the answering of two questions: First, whether there really are two different sizes of spermatozoa produced in approximately equal numbers, and second, whether the centrifuging tends to separate those two sizes. The answer to the first and more fundamental question is not certain. One of the four curves of normal spermatozoa is distinctly bimodal, one is distinctly unimodal, and the other two are broader at the top and narrower in the middle than the theoretical curve but agree with it well at the base. That is the result which would be given if the curve were a compound one composed of two equal normal curves whose modes were not very far apart. Since the size difference between the X-containing and Y-containing spermatozoa is expected to be very small (*1*), these flat-topped curves agree very well with the idea that there really are two sizes of rabbit spermatozoa, but that the means of those two sizes are too close together for the modes to show separately in the curves. All three of the curves of centrifuged spermatozoa are unimodal, and the two for which there is a control (figs. 2 and 3) seem to indicate in both cases that it is the larger spermatozoa which were separated out both to the inside and to the outside of the tube.

Those spermatozoa which were plainly distorted or broken were not measured, but the distortion of many of them may have been so slight that it could not be detected by inspection. Since the answer to the two principal inquiries is concerned only with the average distribution of the two classes of spermatozoa, the extreme variants have very little importance in this problem. Therefore, in figuring the goodness of fit of the actual results to the theoretical, the only classes considered were those in which the theoretical number exceeded six. This arbitrary limit includes the great majority of the individuals and gives a fair indication of the goodness of fit of the actual distribution to the expected distribution without giving undue importance to any chance variation where the expectation is small. It will be noticed that the probability is fairly high for all of the centrifuged slides as compared to the normal ones, there being only one normal one with a value as high as either of the three centrifuged slides. This indicates that the centrifuged samples are more homogeneous with respect to head length of spermatozoa than untreated samples are.

The general conclusions to be drawn from the microscopic work on the rabbit spermatozoa are that there are indications of a dimorphism in the head lengths but no evidence complete enough to establish it beyond question. The two sizes differ so

slightly that it is uncertain whether centrifuging effects a separation at all; certainly it is not a complete one, and the conditions under which each insemination and centrifuging are performed necessarily vary so much that the necessary adjustments of technique appear to be insurmountable difficulties to the successful control of sex in rabbits by this method even though the theoretical basis of the method may be perfectly sound.

The microscopic work on swine spermatozoa was directed toward answering the same two questions as was the work with the rabbits, but was unaccompanied by any breeding work. The first question, as to the existence of a dimorphism in the spermatozoa head lengths, has already been answered very positively by Wodsedalek (*43*), and since the difference reported between the mean sizes of the two classes was so large it seemed that this was excellent material for seeking an answer to the second question. However, the dimorphism revealed by the examination of untreated spermatozoa in this work was not nearly so distinct as that reported by Wodsedalek. Where the expectation is that the mean size of the larger kind will exceed that of the smaller kind by about 20 per cent, the actual results are that the mode of the larger kind is from 7.1 to 8.6 per cent larger than the mode of the smaller kind. Just what is the reason for the difference in results is not clear. The material used is almost identical, the animals being of the same breed, from the same herd, and one at least being of the same age as the one used by Wodsedalek. The only difference, aside from differences in staining and possible errors in measurement, is that the spermatozoa used in this work were mature spermatozoa from the epididymis of the testicles, or were already ejaculated, whereas Wodsedalek measured spermatozoa in the tubules themselves, using as a criterion of their maturity the fact that they were in the lumen free from the surrounding tubule walls.

However, it seems certain that a dimorphism of spermatozoa does exist, although it may not be as great as was first reported. All three of the curves of untreated spermatozoa show two modes, rather close together and of about equal height; all three are wider at the apex and narrower at the middle than the theoretical, and therefore, although the average figure for their goodness of fit is fairly high, it seems certain from inspection that they really are compounded of two overlapping curves of about equal frequency.

The slides made from the centrifuged material agree in indicating that centrifuging tends to separate the smaller spermatozoa to the inside and the larger spermatozoa to the outside of the centrifuge tube, but that the separation is not complete. All three of the slides made from the inside material are skewed to the left and the one made from the outside is quite strongly skewed to the right. The unusual shape of the curve from slide No. 526 may be connected with the fact that it was made of material from the next to the extreme inside one-sixth of the centrifuge tube instead of the extreme inside, as slide No. 531 was. Slides 519, 531, and 536 are made from the same material, and slides Nos. 522 and 526 are both made from material from the younger boar.

The conclusions which can be drawn from the work on swine spermatozoa are that there is a definite dimorphism in size, but that the difference between the two kinds of fully mature spermatozoa is probably not as great as was reported by Wodsedalek. Centrifuging effects a partial separation, throwing more of the large spermatozoa to the outside and leaving more of the small ones in the inside portions of the liquid. As pointed out in discussing the theory of centrifuging, this is the effect to be expected when bodies of varying size are suspended in a liquid which is slightly less dense than the bodies themselves, and that the spermatozoa are of a slightly greater density than the surrounding liquid is shown by the fact that the majority of the spermatozoa are thrown to the outside in 5 minutes of centrifuging and almost all of them if the centrifuging is prolonged much beyond 10 minutes.

Whether the separation is complete enough or the technique can be made simple enough so that sex control by a method based on these principles ever can be made practical in swine is still an open question. There does not appear to be much economic advantage in being able to control sex in swine, but the scientific interest would be considerable. Even if it can be made practicable in swine, such a method would be restricted to animals in which the X-chromosome constituted a very large and the Y-chromosome a very small percentage of the total chromatin of the cell. That, of course, eliminates a large number of animals, including

man himself, from the list of eligibles. In short, this investigation has not strengthened the belief that there is any method of controlling sex among the higher animals which is practical, simple, and of wide application.

SUMMARY

Centrifugal separation of the male-producing and female-producing spermatozoa was attempted with liquid recovered by means of a catheter from the vagina and uterus of female rabbits which had just been served by normal males. Other females were then artificially inseminated with portions of the centrifuged liquid or with liquid treated in other ways and the sex of the young thus produced was recorded. The only deviation from equality which approached statistical significance was in the sexes of the young produced by females which had been served naturally and then had had as much liquid as possible recovered from the vagina and uterus by means of a catheter. There is no evident explanation of this result, and since the deviation is barely significant statistically it is concluded that this is probably a chance deviation.

Microscopic measurements of the head lengths of a large number of rabbit spermatozoa, both before and after centrifuging, show that a dimorphism probably exists but that the size difference between the two groups is very small. It is not certain whether centrifuging has had any effect upon the distribution of sizes.

Similar measurements of swine spermatozoa have shown the existence of a dimorphism in size, but not as great a difference as was reported by Wodsedalek. Centrifuging effected a partial separation, tending to throw more of the larger spermatozoa to the outside of the tube and leaving more of the smaller spermatozoa near the inside.

Sex control by centrifugal separation of male-producing and female-producing spermatozoa seems possible for species where the difference in the sizes of the X-chromosome and the Y-chromosome is a considerable percentage of the total amount of chromatin in the spermatozoa. Whether such a method can be made practicable even for the most favorable species depends upon whether the technique can be simplified and made dependable.

There is nothing in this work which can be regarded as an argument against the chromosome theory of sex, but, on the other hand, there is no new evidence in support of it. The failure to control sex in rabbits may be tentatively attributed to the impossibility of refining the technique to the point where advantage could be taken of the very small difference which exists between the X-chromosomes and the Y-chromosomes of the rabbit.

Spermatozoa retained their fertilizing ability as long as 24 hours in vitro at low temperatures and retained their motility as long as 60 hours in vitro at moderately low temperatures and up to 200 hours when kept in an excised testicle on ice.

Coition is not necessary to ovulation in the rabbit.

The chances of a female becoming pregnant when inseminated were increased by diluting the fluid used, but were decreased by holding it some time at a low temperature.

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CORRELATED INHERITANCE OF BOTANICAL CHARACTERS IN BARLEY, AND MANNER OF REACTION TO HELMINTHOSPORIUM SATIVUM [1]

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INTRODUCTION

The identification of linkage groups and their relation to chromosome numbers is a subject of general genetic interest. In only one organism, Drosophila, have all of the linkage groups been thoroughly worked out. Studies are being made of other organisms, however, with the idea of identifying the chromosomes by the behavior of the factors which they carry. Much has been learned of the factor relations in maize, largely from the efforts of Emerson and his coworkers. It is obviously a more difficult task to establish 10 linkage groups, which is the number of chromosome pairs in corn, than it is to establish 4 such groups, as in Drosophila. For linkage studies, an organism with a small number of chromosomes is desirable and from this standpoint, perhaps, barley offers as favorable material as can be found among the crop plants. Furthermore, barley has a number of easily distinguishable characters.

Through the introduction of new plant varieties from foreign countries and as a result of extensive hybridization studies, new characters are frequently being made available, and some of these have considerable economic importance. A 6-rowed variety of barley with smooth awns and resistance to *Helminthosporium sativum* has been developed and promises to take the place of the best 6-rowed varieties now generally grown in Minnesota. No such 2-rowed variety is known, however. Since there is a demand for 2-rowed barleys in certain sections of Minnesota, the production of a 2-rowed variety with smooth awns and other desirable characters is of some economic importance.

REVIEW OF LITERATURE

A somewhat comprehensive review of literature on barley inheritance was made by Hayes and Garber (*8*).[3] The literature review in the present paper indicates the important work on barley inheritance in its various phases, but the discussion of that work has been somewhat limited on account of lack of space.

CYTOLOGICAL STUDIES

The chromosome number for barley was reported first by Nakao (*13*) and later by Ubisch (*23*, v. *25*). Nakao used a variety of *Hordeum distichon* and found the somatic number of chromosomes to be 14. The same number of chromosomes was found by Ubisch in varieties of *H. distichon* and *H. vulgare*.

INHERITANCE OF DIFFERENTIAL CHARACTERS

Fertility of the lateral florets.—The results obtained from crosses of 2-rowed with 6-rowed varieties vary. Hayes and Garber (*8*) state that "the most frequent result is an intermediate condition in F_1 in which the lateral florets are awned, but produce little or no fruitfulness. In F_2 a 1:2:1 ratio of 6-rowed, intermediate, and 2-rowed forms is obtained. Six-rowed and 2-rowed forms breed true to these respective characters in later generations. Results of this nature can easily be explained on a single main factor difference."

Some crosses of 6-rowed with 2-rowed forms yield different results from the above, however, as was shown by Harlan and Hayes (*6*). The 6-rowed parent carried two factors for fertility of the laterals, one for 6-rowed and another for intermedium which is hypostatic to the 6-rowed factor. There were indications that sometimes modifying factors were present which affected the degree of fruitfulness in the laterals of the intermedium form. Results of some crosses

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[3] Reference is made by number (italic) to "Literature cited," p. 933.

indicated a single-factor difference between intermedium and 6-rowed. Crosses of deficiens with 2-rowed also showed a one-factor difference.

Arlington awnless, classed by Wiggans (*26*) as an intermedium, has been used by Saunders and Moe (*18*) as the female parent in a series of crosses. Arlington awnless×6-rowed bearded produced in F_1 an intermediate type and in F_2 Arlington awnless, intermediate, and 6-rowed in a 1 : 2 : 1 ratio. Arlington awnless×2-rowed hooded produced in F_1 a type resembling the female parent for fertility of the florets in the lateral spikelets but with the florets of the medians hooded. In the second generation the plants were grouped into four classes: I. 2-rowed hooded; II. 6-rowed, or approximately 6-rowed, without awns on the florets of lateral spikelets but with hoods on the florets of the median spikelets; III. 2-rowed bearded with occasionally a few kernels present in the lateral rows; and IV. Arlington awnless type. Plants from Class I bred true in F_3 for the 2-rowed condition but some families segregated for hoods and awns. The plants from Class II differed in the results they gave in F_3. Plants from Class III bred true for 2-rowed bearded. Plants from Class IV were of two types, one bred true for the Arlington awnless type and the other gave Arlington awnless, 2-rowed bearded, and intermediates.

Awns and hoods.—According to Hayes and Garber (*8*), Tschermak obtained results showing a single-factor difference between awnless and hooded forms, with the awnless character dominant. In crosses of hooded varieties with awned varieties the hooded condition tended to be dominant, the ratio in F_2 being 3 hooded to 1 awned. The same results were obtained by Thatcher.

In a series of studies, Ubisch (*23*, *24*) found an interesting relation between the hooded and awned characters. He considered a spike as long-awned when the awns were 6 cm. long or longer, and as short-awned when the awns were shorter than 6 cm.

In later studies Ubisch (*24*) crossed awnless varieties with short-awned and in some cases obtained an awnless F_1 type and in others a hooded F_1 type. The results of the later generations have not been published.

Articulation of rachis.—In some varieties of barley, particularly of the species *Hordeum spontaneum*, the rachis is articulate and the heads shatter quite easily. A one-factor difference between the articulate and the nonarticulate rachis was found by Schiemann (*21*) in a cross in which *H. spontaneum* was used as one of the parents. Articulate types were obtained by Ubisch (*23*, *v. 17*) from crosses in which both the parent varieties were nonarticulate. The ratio of plants with articulate rachis to plants with nonarticulate rachis was approximately 9:7 in the F_2 generation. In one cross the articulation was as pronounced as that of *H. spontaneum*, but in the other crosses was less pronounced.

Dense and lax spike.—The terms dense and lax, as used here, refer to the length of the rachis internode. In dense varieties the internode is short, and in the lax varieties long. The terms lax and dense are relative, since homozygous forms apparently exist (*6*) with small gradations all the way from very dense to very lax. Several factors for density of the rachis were shown to be present in one cross, while other crosses differed apparently by only a single differential factor. Ubisch (*23*, *v. 17*) considered the forms with internode length of 3.5 mm. or greater as lax and with internode of less than 3.5 mm. as dense. With this grouping he obtained one main factor difference for dense versus lax. He assumed other factors with lesser effect were present in some crosses.

Rough awn and smooth awn.—In some varieties of barley the awn has sawlike teeth from base to tip. Various types of teeth were described by Vavilov (*25*), who made a careful morphological study of the teeth on a number of varieties. He also found various degrees of smoothness but failed to find any varieties which lack the teeth on the entire length of the awn. Crosses of rough-awned with smooth-awned varieties were first studied by Harlan (*5*), who found a single factor difference between rough awn and smooth with rough dominant. Vavilov obtained smooth-awned types in the F_2 generation from crosses in which both the parent varieties were rough-awned. In crosses of rough-awned varieties with smooth the F_1 plants were always rough-awned. Crosses were made between rough-awned and smooth-awned varieties at the Minnesota Experiment Station (*10*). The awns of the F_1 plants were rough. In the F_2 generation the ratio of rough to smooth awned plants was approximately 3:1. Third-generation lines from F_2 plants classed as rough-awned varied in their breeding behavior. Some lines produced only rough-awned plants, some produced both rough and smooth, and others

contained all the types of F_2. The results are explained on the basis of one main factor difference between rough awn and smooth awn.

TEETH ON THE GLUMES.—Three kinds of teeth on the glumes of barley are described by Ubisch (*23, v. 17*). Teeth of type (*a*) are visible to the naked eye, type (*b*) are seen by the aid of a hand lens, while type (*c*) can be seen only with a microscope. In crosses of type (*a*) with a nontoothed variety an intermediate toothed condition was obtained in F_1, and in the F_2 there were 3 toothed and intermediate forms to 1 nontoothed.

HULLED AND NAKED SEED.—The naked condition of the seed seems to be a simple recessive to the covered. Hayes and Garber (*8*) cite the results of Gaines and also Thatcher in which the naked condition behaved as a simple recessive to the covered. Ubisch (*23, v. 20*) obtained results which indicated that the hulled condition was not completely dominant, although the results could be explained on a single factor basis.

DWARF FORMS.—Harlan and Pope (*7*) describe a dwarf barley which measured only about 50 cm. from the crown of the plant to the tip of the awns. The dwarf form behaved as a simple recessive to the normal barley. On the other hand, a dwarf was obtained by Miyazawa (*12*) which tended to be dominant to the normal. The homozygous dwarfs were sometimes so small as to escape notice and were sterile. Occasionally a dwarf plant died in the seedling stage. A single factor difference explains the results obtained.

CHLOROPHYLL DEFICIENCES.—Seedling types deficient in chlorophyll have been found in several of the cereals, so it is not surprising to find that they also occur in barley. They have been observed in two Minnesota varieties, but their mode of inheritance has not been studied. Hallquist (*3*) obtained a chlorophyll-defective barley strain which is dependent on temperature for its expression. At 0° to 10° C. no chlorophyll develops, at 12° to 15° some green color develops but the seedlings are very yellow, while at 20° the seedlings are normal green in color almost from the time of their emergence. The defect behaves as a simple recessive to the normal green. Back crosses of the heterozygotes to the normal green gave results which differed depending on whether the normal was used as the female or as the male parent. The results are explained by assuming a single factor difference between the normal and the defective and a partial elimination of the male gametes which carry the recessive factor.

Six different chlorophyll defects are described by Nilsson-Ehle (*14, 16*), three whites, two yellows, and a chlorina. The three whites have been shown by breeding tests to be different genotypically. The yellows have not been tested thoroughly but differ phenotypically. The chlorina corresponds to the virescent types in corn in that under the proper cultural conditions it lives and produces seed. Each of the abnormal types behaves as a simple recessive to the normal green.

A mutation was discovered by Kiessling (*11*) which differed strikingly from the parent variety in many morphological and physiological characters, including a light green color of the foliage. The deviations from the normal, other than that of chlorophyll, are considered by Kiessling as incidental to the chlorophyll deficiency. The abnormal type, like those studied by Nilsson-Ehle, is a simple recessive to the normal.

DISEASE RESISTANCE.—Nilsson-Ehle (*15*) made a study of the inheritance in barley of the manner of reaction to the nematode disease caused by *Heterodera schachtii*. The cross Chavalier × Gold, immune and susceptible varieties, respectively, gave F_1 plants which were immune and the segregation in F_2, as shown by the breeding behavior in F_3, approximated a 3:1 ratio. These results show susceptibility to be a simple recessive to immunity.

Studies have been made by Hayes et al (*10*) on the inheritance of the manner of reaction in barley to *Helminthosporium sativum*. A careful study was made in a cross of a resistant variety with a highly susceptible variety. One hundred and twenty-four F_3 lines were grown under epidemic conditions in comparison with the parent varieties. The number of homozygous resistant and susceptible lines was determined from a test of F_3 and F_4 families. It was pointed out that the results could be explained by assuming two pairs of factors to be involved. The presence of both factors leads to the production of highly resistant forms while the absence of both gives highly susceptible forms.

COLOR IN GLUMES.—Black color in the glumes behaves as a dominant to white, and in all results so far obtained a single factor difference exists between black and white. Purple color in the glumes is also dominant to white, and

in crosses of purple with white the F_2 generation shows a ratio of 3 purple to 1 white.

LINKAGE RELATIONS.—Although considerable work has been done on inheritance in barley, comparatively few cases of linkage have been shown definitely. Ubisch (*23 v. 17, 20*) showed a correlation between the internode length of the rachis and the length of the awn. In crosses of varieties with long awns and lax spike with varieties having short awns and dense spike the F_2 results indicated a gametic ratio of 4 or 5 to 1. In later studies (*23, v. 25*) he obtained a correlation between the length of awn and naked seed. The gametic ration was 1:6. A gametic ratio of 1:5 was obtained in the cross of varieties having lax spike and naked seed with varieties having dense spike and covered seed. From these results, Ubisch reached the following conclusions: Factors for the length of awn and the length of internode are linked with 16.7 per cent crossovers; factors for the length of awn and covered versus naked seed are linked with 14.3 per cent crossovers; and factors for internode length and for covered versus naked seed are linked with 16.7 per cent crossovers. These results seem inconsistent with the linear theory for the arrangement of genes in the chromosome. Several factors have been found, however, producing varying densities of the spike. Because of this fact and the absence of a careful statistical analysis one is hardly justified in drawing definite conclusions from these results. Ubisch (*23, v. 17*) also found a linkage between the factor for 6-rowed with one of the factors for teeth on the glumes. The crossover percentage was 16.7.

A correlation was found by Vavilov (*25*) between the factor for rough awn and the factor for naked seed. The intensity of the linkage was not worked out.

Nilsson-Ehle (*16*) showed that one of the factor pairs for green versus white seedlings (factor pair Cc) is correlated with the factor pair for green versus chlorina (factor pair Ff). In the cross CcFF with CCff he studied the progeny of the combination cF×Cf. In F_2 he expected a ratio of 9 green : 3 chlorina : 4 white, and obtained 8.44 : 3.94 : 3.62, respectively. Instead of the normal 1 : 2 : 2 : 4 ratio in F_3 from green F_2 plants, he obtained no families which bred true for green, 9 families which segregated into green and chlorina, 12 which segregated for green and white seedlings, and 126 families which produced all types. He suggested linkage with about 10 per cent crossing over as the best explanation.

In the foregoing review a number of character pairs are considered. It seems, however, that little has been done to establish the relation of these character pairs to each other. One of the best studies from the standpoint of the relation of factors for several character pairs is that carried on at the Minnesota station in cooperation with the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and cited by Hayes and Garber (*8*). Four character pairs were studied in one cross: Hooded versus awned, 2-rowed versus 6-rowed, black versus white seed, and hulled versus naked seed. These four character pairs were shown to be independently inherited or, in other words, to represent four linkage groups.

According to the work of Vavilov (*25*), one of the factors for dentition of the awn is correlated with the factors for hulled versus naked seed. The results obtained by Ubisch (*22*) indicated that this group also contains a factor for length of awn and one for length of rachis internode.

In the present study the various character pairs are considered in their relation to each other with an idea of establishing a linkage group for each chromosome pair.

MATERIAL USED

All varieties used in these studies are known to be pure lines:

Svanhals is a variety of *Hordeum distichon*. It has white glumes, rough awns, headed about July 6, and is highly resistant to the spot blotch disease caused by *Helminthosporium sativum*.

Lion is a variety of *Hordeum vulgare*. It has black glumes, smooth awns, headed about June 21, and is highly susceptible to the spot blotch disease.

Manchuria, I–16–66, is a selection from Manchuria, a variety of *Hordeum vulgare*. It has white glumes.

Hordeum deficiens steudelii, as its name indicates is a *deficiens* variety. It has black glumes and the lemma and palet of the florets in the lateral spikelets are developed slightly or not at all.

Hordeum intermedium cornutum is a variety of *intermedium* (*4*) with practically complete fertility in the florets of the lateral spikelets. The florets of the median spikelets are hooded. It is hulless and has white seeds.

The results given here are from the crosses Svanhals×Lion and *Hordeum deficiens steudelii*×Manchuria. The crosses were made at University Farm, St. Paul, Minn., in 1921 and the F_1

generations were grown in the United States Department of Agriculture greenhouse at Arlington Experiment Farm, Rosslyn, Va., in the winter of 1921–22 and also in the plant breeding nursery at University Farm in 1922. Second generations were grown at University Farm in 1922 and 1923. Progeny from the 1922 F_2 generation plants were tested for breeding behavior in 1923.

EXPERIMENTAL RESULTS

In the presentation of the experimental results the data will be considered only for those characters for which the results seemingly warrant rather definite conclusions.

CYTOLOGICAL STUDY

So far as the writer is aware, no other work has been done in America on the chromosome numbers in barley species. The reports of Nakao and Ubisch are incomplete, not all cultivated species being included.

The photomicrographs included here are from transverse sections of the root tips of barley. The killing fluid used is a modification of Bouin's (used first by Allen (*1*) and recently used successfully by Sax [4]) on wheat material at the Maine Agricultural Experiment Station. The sections were cut 5 μ thick and stained with Haidenhain's iron alum haematoxylin.

Since crosses of varieties of the four cultivated species show no sterility and the chromosome number of some species was shown to be 14 in the somatic cells, one naturally would expect to find the same number in all cultivated species. A variety of each of the species *Hordeum vulgare*, *H. intermedium*, *H. distichon*, and *H. deficiens* is included in the cytological study. The chromosome number is the same for all varieties studied—that is, 14 in the somatic cells. This fact leads one to conclude that seven linkage groups will be found. On the basis of chromosome numbers, all varieties of these four species belong in one group (pls. 1 and 2).

As the writer is interested mainly in chromosome number from the standpoint of linkage groups, and since the photomicrographs show the number clearly, no drawings were made. One peculiarity of structure seems worth noting, however (pl. 1, A and B), and that is a more or less definite area near the end of a chromosome which does not take the stain. That the piece beyond the hyaline area is a part of the chromosome back of it is indicated by the fact that the small piece is always at the end and in line with the main part of the chromosome. When the chromosomes are long and thin (as iu pl. 1, C), usually two chromosomes of the group have this disjointed appearance.

INHERITANCE OF DIFFERENTIAL CHARACTER PAIRS

FERTILITY OF LATERAL FLORETS.—In the cross *H. deficiens steudelii* × *Manchuria* the F_1 plants which were grown in the greenhouse could not be distinguished from the *deficiens* parent. Under field conditions, however, many F_1 plants show slight development of the glumes and palets in the laterals, and in some seasons the F_1 may be quite accurately distinguished from the homozygous deficiens. In the F_2 generation, grown in 1922, the plants could be grouped quite easily into the three types, *deficiens*, intermediate, and 6-rowed, and approximated closely a 1:2:1 ratio (Table I).

TABLE I.—*Classification of F_2 plants for the characters, deficiens and vulgare in the cross Hordeum deficiens steudelii* × *Manchuria (vulgare); 1922*

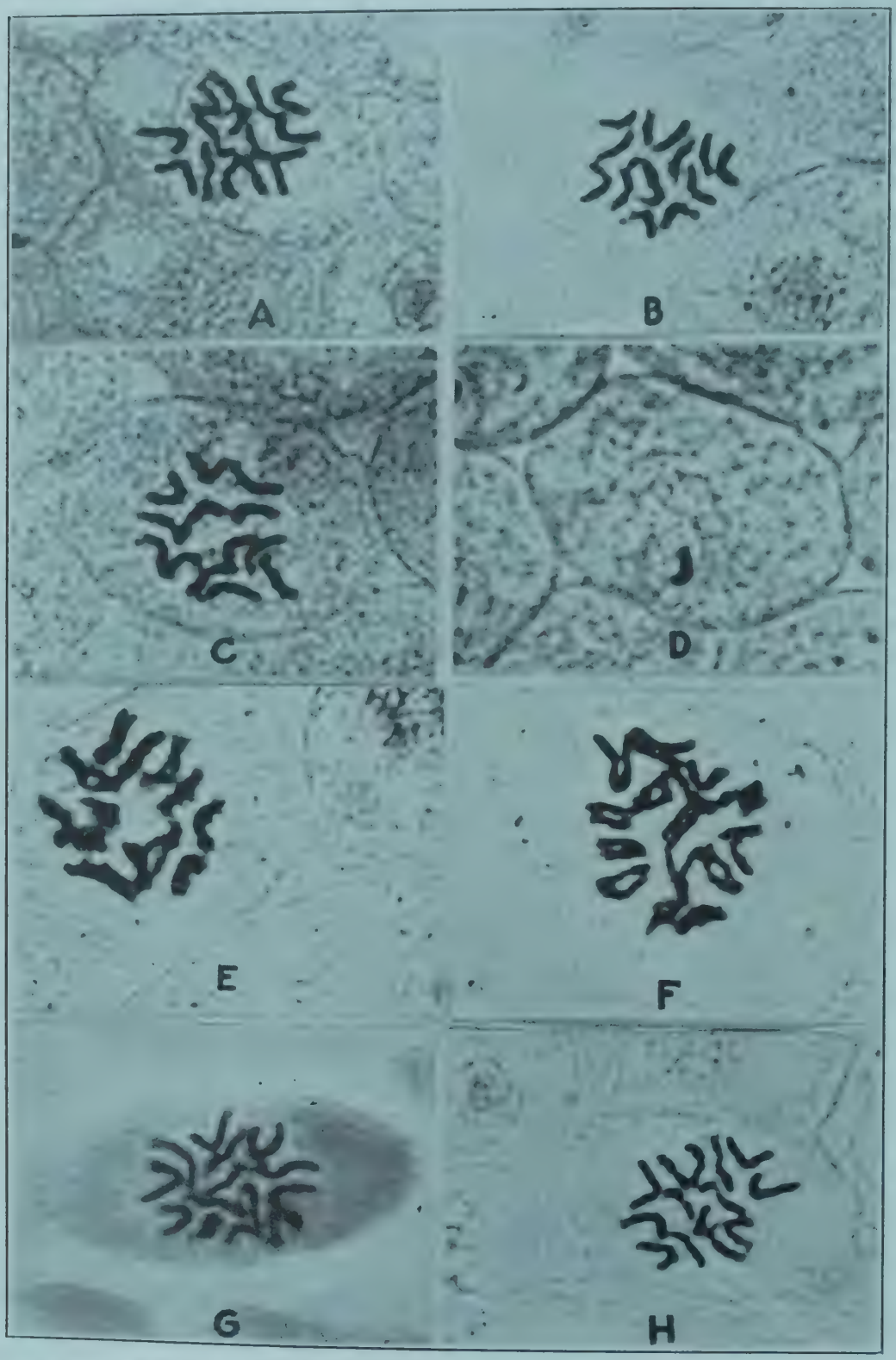
Class			Total
Vulgare	Intermediate	Deficiens	
84	191	80	355 observed.
89	178	89	356 calculated on 1 : 2 : 1 basis.

$X^2 = 2.14$. $P = 0.3476$.

All plants classed as 6-rowed in F_2 bred true for this character in F_3 (Table II). All of the *deficiens* plants tested bred true, while all of the plants classed as intermediate segregated with one exception.

The heterozygotes and deficiens could not always be distinguished in the F_2 generation which was grown in 1923, but when these two groups are considered together the resuts show a good fit for a 3 : 1 ratio of deficiens and intermediates to 6-rowed (see Table III). These results show conclusively that the characters 6-rowed and de-

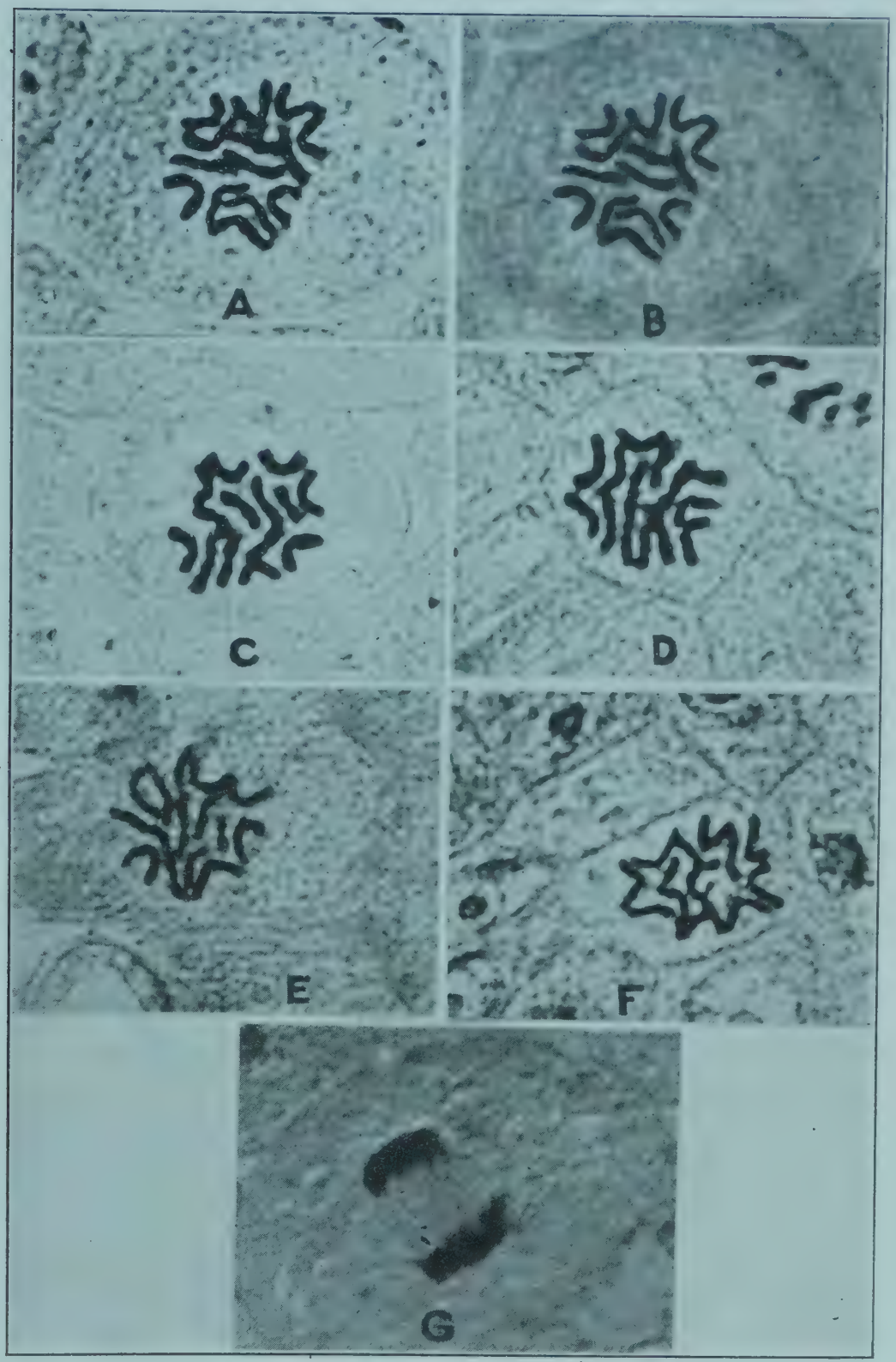
[4] Sax kindly furnished the writer with the details of his method of using Bouin's killing fluid.



A to F.—Cells from the root tips of Manchuria, a variety of *Hordeum vulgare*. In A, 14 chromosomes are clearly visible. In B, 12 chromosomes are clearly seen; the other two overlap at the ends and appear to be one big chromosome. C and D are different sections of the same cell, 13 of the chromosomes appearing in C and the other one in D. In E may be seen the beginning of the separation of the daughter chromosomes. In F is seen a later stage than that in E. × approximately 1,400

G and H.—These are from the root tips of *H. Intermedium cornutum*. Each contains 14 chromosomes. × approximately 1,400

(Material was killed in modified Bouin's killing fluid and stained with Haidenhain's iron alum haematoxylin. Sections were cut transversely in thickness of 5 microns. Unless otherwise stated, a K–1 filter was used in taking the photomicrographs)



A to D are from the root tips of Svanhals, a variety of *Hordeum distichon*. A and B are of the same cell. B was taken using a ground-glass filter with a 10-minute exposure, to bring out all details clearly

E to G are from the root tips of *Hordeum deficiens steudelii*. In E the 14 chromosomes are clearly visible. F shows only 13 chromosomes, but these are arranged in a peculiar pattern

(Material was killed in modified Bouin's killing fluid and stained with Haidenhain's iron alum haematoxylin. Sections were cut transversely, in thickness of 5 microns. Unless otherwise stated, a K–1 filter was used in taking the photomicrographs)

TABLE II.—*Behavior of F_3 lines from the three F_2 classes deficiens, intermediate and vulgare, in the cross Hordeum deficiens steudelii × Manchuria; 1923*

Type of plant	Number of plants tested	Classes in F_3			Number of families
		Deficiens	Intermediate	Vulgare	
Vulgare	20			×	20
Deficiens	32	×			32
Intermediate	50	×	×	×	49
		×			1

ficiens differ in a single genetic factor. The 6-rowed character may be considered to be due to the factor V, the 6-rowed condition being developed only when the plant is homozygous for this factor. The genotype for deficiens would then be vv.

TABLE III.—*Classification of F_2 plants for the characters deficiens and vulgare in the cross Hordeum deficiens steudelii × Manchuria; 1923*

Class			Total
Vulgare	Intermediate	Deficiens	
147	206	255	608 observed.
152	304	152	608 calculated on a 1 : 2 : 1 basis.
147	461		608 observed.
152	456		608 calculated on a 1 : 3 basis.

Deviation=5. P. E.=7.2.

Svanhals crossed with Lion gave F_1 plants which resembled the 2-rowed parent, but the glumes of the laterals were more pointed, and in some cases bore short awns. In the second generation the plants were grouped into four groups, 6-rowed, 2-rowed, intermedium, and intermediate. Only the 2-rowed and 6-rowed groups were tested thoroughly in F_3. All of the plants classed as 6-rowed bred true for that character. Fully two-thirds of the plants classed as 2-rowed in F_2 segregated in F_3; some produced 2-rowed, intermediates, and 6-rowed, some 2-rowed and intermedium, while others produced all types of F_2. The intermediums obtained in F_2 are of the same type as those obtained by Harlan and Hayes (*6*). Strains of this low fertility intermedium were obtained from the cross Svanhals × Lion, which bred true in F_3. Since all groups of F_2 plants were not tested thoroughly in F_3, a complete factor explanation will not be attempted. It should be pointed out, however, that the 6-rowed F_2 plants constitute approximately one-fourth of the entire number of plants (see Table IV), and that lines which segregated for 2-rowed versus 6-rowed gave a close approximation to a ratio of three 2-rowed and intermediates to one 6-rowed (see Table V). These facts, together with the appearance of a low fertility intermedium, indicate a two-factor explanation as used by Harlan and Hayes (*6*). These results prove that the low fertility intermedium factor may be carried by a 6-rowed variety and that the presence of this factor does not modify the expression of the factor for the 6-rowed condition. Two-rowed lacks the factors for intermedium and 6-rowed. Apparently the factor pairs which differentiate 6-rowed versus 2-rowed and intermedium versus 2-rowed are independently inherited.

TABLE IV.—*Distribution of plants for characters 2-rowed and 6-rowed in the F_2 generation in the cross Svanhals × Lion*

Year	Two-rowed and intermediates	Six-rowed	Total
1922	940	319	1,259 observed.
	944.25	314.75	1,259 calculated on a 3:1 basis.
	--------	4.25	Deviation.
	--------	10.30	Probable error.
1923	368	112	480 observed.
	360	120	480 calculated on a 3:1 basis.
	--------	8	Deviation.
	--------	6.4	Probable error.

TABLE V.—*Distribution of plants for the characters 2-rowed and 6-rowed in the F_3 lines which segregated, giving only these types and intermediates; cross Svanhals × Lion*

Two-rowed and intermediates	Six-rowed	Total
33	12	45.
27	13	40.
35	9	44.
34	13	47.
129	47	176 total observed.
132	44	176 calculated.
	3	Deviation.
	3.87	Probable error.

TABLE VI.—*Distribution of plants for the characters black glume and white glume in the F_2 generation of the crosses Hordeum deficiens steudelii×Manchuria and Svanhals×Lion*

Year grown	Black-glumed	White-glumed	Total	Cross
1922	263	92	355 observed	H. deficiens×Manchuria.
	266. 25	88. 75	355 calculated	
		3. 25	Deviation	
		5. 5	Probable error	
1923	470	138	608 observed	
	456	152	608 calculated	
		14	Deviation	
		7. 2	Probable error	
1922	871	283	1, 154 observed	Svanhals×Lion.
	865. 5	288. 5	1, 154 alculated	
		5. 5	Deviation	
		9. 9	Probable error	
1923	360	120	480 observed	
	360	120	480 calculated	
		0	Deviation	
		6. 4	Probable error	

TABLE VII.—*Behavior of F_3 lines from plants classified in F_2 as black glumed, in the crosses Hordeum deficiens steudelii×Manchuria and Savanhals×Lion*

Breeding true for black	Segregating in 3:1 ratio	Total	Cross
24. 0	41. 0	65. 0 observed	H. deficiens steudelii × Manchuria.
21. 7	43. 4	65. 1 calculated	
	2. 4	Deviation	
	2. 6	Probable error	
25. 0	58. 0	83. 0 observed	Svanhals×Lion.
27. 7	55. 4	83. 1 calculated	
	2. 6	Deviation	
	2. 9	Probable error	

BLACK VERSUS WHITE GLUMES.—The glumes of plants of the F_1 generation in the crosses *Hordeum deficiens steudelii*×Manchuria and Svanhals ×Lion are as black as the colored parent. In the second generation the ratio of plants with black color to plants that are white is very close to 3 : 1 (see Table VI). All white F_2 plants bred true for white in F_3, as expected. Sixty-five F_3 lines from one cross and 83 from the other were grown from plants classed as black in F_2. The deviation from the expected 1 : 2 ratio is less than the probable error in each case (see Table VII). Apparently black and white differ by a single factor pair.

ROUGH AWN VERSUS SMOOTH.—Rough-awned varieties of barley are very disagreeable to handle, and for this reason the smooth-awn character is of considerable practical importance for those regions where awned varieties are preferred. The cross Svanhals× Lion gave F_1 plants with awns as rough as those of the Svanhals parent. The F_2 plants were easily classified into two groups, the first with awns entirely rough and the second with awns partially or almost entirely smooth. All plants which had awns with any degree of smoothness were studied carefully, the awns being examined under magnification. An index for smoothness was derived in a similar manner as in previous studies at the Minnesota station (*10*) which briefly is as follows: The total length of the awn in centimeters is divided by the portion of the awn from the tip where there are teeth of large size at frequent intervals. The higher the index of smoothness the smoother the awn, and vice versa. A rough awn has an index of 1 while the Lion parent used in this study has an average index slightly above 3. Some plants of Lion have a few scattered teeth, about half the distance from the tip to the base of the awn, but these teeth are usually of slightly reduced size.

An arbitrary division was made at an index of 2 and plants with a higher index were called smooth while plants with a lower index than 2 but with some degree of smoothness were called

"intermediate-smooth." With this grouping the distribution of plants in F_2 indicates a 2-factor difference for roughness of awn between Lion and Svanhals. The factor R, when present, gives rough awn. The other factor, S, is hypostatic to R and in the absence of R produces intermediate-smooth awn. The double recessive, rrss, is a smooth awn of the Lion type. With this explanation the ratio in F_2 would be 12 rough : 3 intermediate-smooth : 1 smooth. The observed distribution of plants in the F_2 generation grown in 1922 does not fit the calculated distribution very well, a deviation as large as that encountered being expected only about 2 times out of 100 trials (Table VIII).

The fit is better for the distribution of F_2 plants which were grown in 1923, but still the deviation is rather large. The reason for these deviations will be seen by examining Tables IX, X, and XI.

The behavior of the F_3 lines from plants classed in F_2 as rough is in close agreement with expectation, X^2 being 1.4 (P=0.7097). Of 37 F_3 lines from plants classed in F_2 as intermediate-smooth, 7 gave rough-awn plants in addition to intermediate-smooths, and smooths. However, the discarding of these families should not affect the expected 1:2 ratio of homozygous to heterozygous intermediate - smooths. Disregarding these 7 families, the other 30 families give a close approximation of a 1:2 ratio for homozygous intermediate-smooth to heterozygous (see Table X).

Thirty-four F_3 lines were grown from plants classed as smooth in F_2. Of these lines, 2 gave rough-awn plants, intermediate-smooth awn and smooth; while 8 gave intermediate-smooth and smooth. The other 24 lines vary in their indices from 2.85 to 3.61 (see Table XI). When a correction is made of the F_2 distribution of 1922, on the

TABLE VIII.—*Distribution of plants for the characters rough awn, intermediate-smooth awn, and smooth awn. F_2 generation of the cross Svanhals × Lion.*

Year grown	Character of awn			Total
	Rough	Intermediate-smooth	Smooth	
1922	908	251	100	1,259 observed.
	948	237	79	1,259+ calculated on 12:3:1 basis.
				X^2=8.1.
				P=0.0174.
1923	362	79	39	480 observed.
	360	90	30	480 calculated on 12:3:1 basis.
				X^2=4.0.
				P=0.1353.
Assumed genotype per 16.	1 RR SS ; 2 Rr SS 2 RR Ss 4 Rr Ss 1 RR ss 2 Rr ss	1 rr SS 2 rr Ss	1 rr ss	

TABLE IX.—*Summary of the behavior of F_3 lines from plants classified in F_2 as rough awn; cross Svanhals × Lion*

Behavior of lines	Number of families		Summary of lines segregating	P. E. or X^2
	Calculated	Observed		
Breeding true for rough	21.1	23		
Segregating rough : intermediate-smooth	10.6	9	290 : 71 observed 270 : 90 calculated	5.6
Segregating rough : smooth	10.6	8	262 : 81 observed 258 : 86 calculated	5.4
Segregating rough : intermediate-smooth : smooth	21.2	24	732 : 185 : 63 observed 732 : 183 : 61 calculated	X^2=1—
Total number families	63.6	64		

basis of the breeding behavior of F_3 lines, the observed frequency fits the calculated very well, P being 0.5278 (Table XII).

After two years' experience the writer is convinced that it is a simple matter to separate rough-awn plants from smooth and intermediate-smooth awn plants. The breeding test, however, is the only sure way to separate the intermediate-smooth and smooth-awn types.

Although a two-factor explanation is given to the results, considerable variability exists in the average indices of the F_3 lines breeding true for smooth. Row 75, for example, with an index of 3.61 ± 0.06 (see Table XI), differs markedly from row 63 which has an index of 2.85 ± 0.05. The difference between these two lines is 0.76 ± 0.08, which is certainly significant in the light of its probable error. These minor variations suggest the presence

TABLE X.—*Summary of the behavior of F_3 families from plants classed as intermediate-smooth in F_2; cross Svanhals × Lion*

Behavior of lines	Number of families		Summation of lines segregating	P. E.
	Calculated	Observed		
Breeding true for intermediate-smooth	10	12		
Segregating intermediate-smooth : smooth	20	18	558 : 176 observed 551 : 183 calculated	7.9

Seven lines gave rough, intermediate-smooth, and smooth.

TABLE XI.—*Behavior of F_3 families from plants classified in F_2 as smooth in comparison with the Lion parent; cross Svanhals × Lion*

F_2 Plant No.	1923 Row No.	Index of F_2 plant	Frequency for awn index of F_3 family				Total number plants	Average awn index	Standard deviation
			2.1	3.0	3.9	4.8			
Lion			6	25	12		43	3.13±0.06	0.57±0.04
Do			9	21	8		38	2.98± .07	.60± .05
Do			4	31	5	1	41	3.06± .05	.50± .04
Do			11	19	12	2	44	3.01± .08	.75± .05
Do			5	31	9		45	3.08± .05	.50± .04
Do			8	27	5		40	2.93± .05	.51± .04
Do			4	27	10		41	3.13± .05	.46± .03
Do			5	22	12		39	3.16± .06	.57± .04
Do			11	22	13	1	47	3.08± .07	.69± .05
Do			7	22	12		41	3.01± .06	.61± .05
Do			11	25	9		45	2.96± .06	.60± .04
Do			9	28	6		43	2.94± .05	.53± .04
Do			5	26	9		40	3.09± .06	.52± .04
Do			8	21	6	1	36	3.00± .07	.64± .05
115–164	63	3.3	11	26	4		41	2.85± .05	.52± .04
115–200	64	3.3	9	23	7		39	2.95± .05	.48± .04
139–172	68	3.3	8	25	11		44	3.06± .06	.59± .04
148–162	70	2.5	11	22	5		38	2.86± .06	.57± .04
148–163	71	3.3	6	25	6		37	3.00± .06	.51± .04
139–126	72	5.0	1	18	13	5	37	3.45± .06	.55± .04
115–195	75	3.3		13	15	6	34	3.61± .06	.49± .04
124–16	76	4.0	2	19	20	2	43	3.43± .06	.54± .04
130–4	77	3.3	2	31	7		40	3.11± .04	.51± .04
130–36	78	3.3	8	18	9	1	36	3.06± .07	.65± .05
130–146	79	4.0	3	18	19		40	3.36± .06	.59± .04
107–87	140	3.3	3	30	6		39	3.07± .05	.43± .03
107–117	141	3.3	4	20	12	1	37	3.24± .07	.62± .05
115–146	142	4.0	6	26	4	1	37	3.00± .06	.55± .04
115–156	143	3.3	11	25	5		41	2.87± .06	.55± .04
124–128	148	4.0	4	26	16		46	3.23± .05	.55± .04
130–129	149	3.3	2	18	15	2	37	3.41± .07	.62± .05
139–35	151	3.3	11	24	5		40	2.86± .06	.55± .04
148–25	153	2.8	8	26	8	1	43	3.04± .06	.61± .04
148–29	154	2.0	4	24	14	1	43	3.25± .06	.59± .04
148–244	155	3.3	9	25	6	1	41	3.09± .05	.52± .04
115–12	159	3.3	1	18	13	4	36	3.50± .07	.65± .05
130–187	162	3.3	5	21	14	1	41	3.24± .07	.63± .05
130–166	65	2.8	4	9	14		27	3.33± .08	.65± .05

TABLE XII.—*Distribution of F_2 plants for the characters rough awn, intermediate-smooth awn, and smooth awn, on the basis of breeding behavior in F_3; cross Svanhals×Lion, 1922*

F_2 class	Distribution on basis of F_3 behavior			Total
	Rough awn	Intermediate-smooth awn	Smooth awn	
	Per cent	*Per cent*	*Per cent*	*Per cent*
Rough awn	100			100
Intermediate-smooth awn	18.9	81.1		100
Smooth awn	5.9	23.5	70.6	100
Observed frequency	908	251	100	1,259
Corrected frequency	961	228	71	1,260
Calculated frequency on 12:3:1 basis	948	237	79	1,264
				X^2=1.33.
				P =0.5278.

of other factors affecting the distribution of teeth on the awn. On account of the difficulty encountered in an attempt to analyze characters of this nature, a more careful study than the one made is not considered feasible at the present time. When all of the linkage groups have been identified, it should be possible to locate easily these minor factors for dentition of the awn.

DATE OF HEADING.[5]—A study was made of the date of heading in 135 F_3 families of the cross Svanhals×Lion. The date of heading for Lion averaged about June 21 while that for Svanhals was around July 6, a difference of 15 days. (See Table XXVI, Appendix.) The F_3 lines proved to be of three kinds, as shown by the distribution for date of heading, two with a unimodal distribution and one with a bimodal. One type was like Svanhals, one like Lion, while the third produced both early and late plants with a preponderance of plants heading early. These three types appeared in approximately a 1:2:1 ratio. (Tables XIII and XXVI. The last named is in the Appendix.) The results show a one-factor difference for date of heading between Lion and Svanhals, early heading tending to be the dominant character.

REACTION TO HELMINTHOSPORIUM SATIVUM.—As already mentioned, Svanhals is highly resistant and Lion very susceptible to the spot blotch disease. For a study of the inheritance of the reactions to this disease, F_3 lines from the cross Svanhals×Lion were used. Seventy lines from plants classed as 6-rowed in F_2 and 65 lines from plants classed as 2-rowed were grown in F_3, making 135 lines in all. Among these lines the characters black and white, as well as rough awn and smooth awn, were represented. Twenty-five seeds of each line were planted in a 5-foot row and two systematically distributed rows were grown of each F_3 line. The parent varities were planted every tenth row. Inoculum was prepared by culturing the pathogene on a cooked wheat and barley mixture. It was sown in the rows at planting, and at heading time the plants were sprayed several times with a water suspension of spores.

TABLE XIII.—*Distribution of F_3 families for date of heading; cross Svanhals×Lion, 1923*

Early	Segregating	Late	Total
33	71	30	134 observed.
33.5	67	33.5	134 calculated on 1:2:1 basis.

X^2=less than 1. P=good fit.

Notes were taken on the infection by the same method as was used in previous studies at the Minnesota station (*10*). By this method a numerical figure is obtained which is an index of the reaction of the line, the higher the figure the more resistant the line, and vice versa. The notes were taken on the lines in the replicate rows without knowing what figure had been given them in the first series. The numerical figures for series one and series two were then correlated (Table XIV). The value of r was found to be $+0.382 \pm 0.050$.

[5] The time of awn emergency is taken as the most convenient criterion of date of heading.

TABLE XIV.—*Correlation of the Helminthosporium figure in series I and II of 135 F_3 families; cross Svanhals×Lion, 1923*

Figure for Series I	Figure for Series II							Total
	9	12	15	18	21	24	27	
9	----	1	----	----	----	----	----	1
12	----	1	7	4	1	----	----	13
15	----	2	11	11	4	3	----	31
18	----	----	6	11	6	5	1	29
21	----	----	5	20	15	5	----	45
24	----	----	1	4	3	5	----	14
27	----	----	1	1	----	----	----	2
Total	----	4	31	51	29	18	2	135

$r = +0.382 \pm 0.050$.

This figure, while not high, shows that definite differences exist between the F_3 lines and that these differences tend to appear alike in both series. The probable error calculated by the "deviation from the mean" method (*9*) was 4.7 per cent. With the probable error of the experiment as 4.7 per cent, the probable error of a difference is 6.6 per cent. If one considers three times the probable error of a difference as significant, then a difference in Helminthosporium reaction to be of importance must be three or more in the more susceptible lines and five or more in the resistant lines.

The numerical figure for Lion was 13.1 and for Svanhals 23.6 (Table XV). The F_3 lines varied in their reactions, some being as resistant as Svanhals while others were as susceptible as Lion. The distribution of the lines fits a normal frequency curve fairly well. From these data alone no definite conclusions can be drawn as to the number of genetic factors concerned in the production of resistance. It is of interest, however, to consider the reaction to Helminthosporium in relation to other characteristics. (See under "Linkage relations.")

INDEPENDENT INHERITANCE

BLACK VERSUS WHITE AND TWO-ROWED VERSUS SIX-ROWED.—In the cross Svanhals×Lion, the character pair 6-rowed versus 2-rowed was shown to be differentiated by a single factor. The same was shown to be the case for the character pair black versus white. Likewise, in the cross *Hordeum deficiens steudelii×Manchuria* the character pairs *deficiens* versus 6-rowed and black versus white were shown each to be differentiated by a single factor pair. The relation of the character pair 2-rowed or *deficiens* versus 6-rowed to the character pair black versus white is shown in Tables XVI and XVII. In the cross *H. deficiens×Manchuria* the factors for the characters *deficiens* and black went into the cross together and in the cross Svanhals×Lion the factor for 6-rowed went in with the factor for black. The results show the character pairs 2-rowed or *deficiens* versus 6-rowed and black versus white to be independently inherited.

TABLE XV.—*Helminthosporium reaction of F_3 lines and parent varieties in the cross Svanhals×Lion*

	Helminthosporium index						Total	Average
	12	15	18	21	24	27		
Svanhals	----	----	----	3	10	1	14	23.6±0.03
Lion	9	5	----	----	----	----	14	13.1±0.02
F_3 families	6	31	42	43	12	1	135	18.6

TABLE XVI.—*Distribution of F_2 plants for the characters deficiens versus six-rowed and black glume versus white. Hordeum deficiens steudelii×Manchuria*

Deficiens and intermediate black	Deficiens and intermediate white	Vulgare black	Vulgare white	Total	Year grown
211	52	60	32	355, observed	1922
200	67	67	22	356, calculated on 9:3:3:1 basis	
				$X^2=8.81$.	
				$P=0.0324$.	
360	110	101	37	608, observed	1923
342	114	114	38	608, calculated on 9:3:3:1 basis	
				$X^2=2.60$.	
				$P=0.4645$.	

TABLE XVII.—*Distribution of F_2 plants for the characters black glume versus white and 2-rowed versus 6-rowed; cross Svanhals×Lion*

Two-rowed and intermediate black	Two-rowed and intermediate white	Six-rowed black	Six-rowed white	Total	Year grown
651	222	221	60	1,154 observed	1922
649	216	216	72	1,153 calculated on 9 : 3 : 3 : 1 basis	
				X^2=2.29.	
				P=0.52.	
280	80	88	32	480 observed	1923
270	90	90	30	480 calculated on 9 : 3 : 3 : 1 basis	
				X^2=1.66.	
				P=0.6504.	

ROUGH VERSUS SMOOTH AWN AND TWO-ROWED VERSUS SIX-ROWED.—Since the data on the roughness of awn for the F_2 of the cross Svanhals×Lion grown in 1923 is close to calculated, it alone will be used in the discussion of correlated inheritance. The factors for 2-rowed and 6-rowed characters are independent of the factors for rough awn, intermediate-smooth awn, and smooth awn (Table XVIII). When the goodness of fit is calculated, the value of P is 0.2301. A deviation as large or larger than this would be expected about once in four trials.

ROUGH VERSUS SMOOTH AWN, AND BLACK VERSUS WHITE GLUMES.—The factors for black and white appear to be independent of the factors for roughness of the awn (Table XIX). A deviation as large as the one obtained would be expected in about half the trials.

LINKAGE RELATIONS

The results so far discussed show that the factors which differentiate *deficiens*, 2-rowed and 6-rowed, black versus white, rough versus smooth awn, and intermediate-smooth versus smooth

TABLE XVIII.—*Distribution of F_2 plants for the characters 2-rowed, 6-rowed, and rough awn, intermediate-smooth awn, smooth awn; cross Svanhals×Lion, 1923*

Two-rowed			Six-rowed			Total
Rough	Intermediate-smooth	Smooth	Rough	Intermediate-smooth	Smooth	
276	60	33	86	19	6	480 observed
270	67.5	22.5	90	22.5	7.5	480 calculated.
						X^2=6.89.
						P=0.2301.

TABLE XIX.—*Distribution of F_2 plants for the characters black glumes versus white and rough awn, intermediate-smooth awn, and smooth awn; cross Svanhals ×Lion, 1923*

Black glumes			White glumes			Total
Rough	Intermediate-smooth	Smooth	Rough	Intermediate-smooth	Smooth	
271	60	28	91	19	11	480 observed.
270	67.5	22.5	90	22.5	7.5	480 calculated.
						X^2=4.24.
						P=0.5178.

awn, are located in different chromosome pairs. A different relation was found to exist, however, between some of these factors and the factors for early versus late heading and the factors for resistance and susceptibility to the spot blotch disease.

TABLE XX.—*Distribution of black-glumed and white-glumed F_3 lines for date of heading; cross Svanhals × Lion, 1923*

Color of glume	Heading date			Total
	Early	Segregating	Late	
Black	7	14	3	24
White	13	26	12	51
Total	20	40	15	75

EARLY AND LATE HEADING IN RELATION TO OTHER CHARACTERS.—Fifty-one white glumed F_3 lines were grown from the cross Svanhals × Lion. Of these 51 lines, 13 bred true for early heading, 26 segregated for early versus late, and 12 bred true for late (Table XX). Of 24 lines breeding true for black glumes, 7 lines bred true for early heading, 14 segregated for early versus late, and 3 bred true for late. These results show the independence of the character pairs early versus late heading and black versus white glumes. The data for the date of heading of the rough-awn and smooth-awn F_3 families indicate here also an independence (Table XXI) although the number of lines is small in each group.

TABLE XXI.—*Distribution of rough-awn, intermediate-smooth-awn, and smooth-awn F_3 lines for date of heading; cross Svanhals × Lion, 1923*

Awn class	Heading date			Total number of families
	Early	Segregating	Late	
Rough	8	7	8	23
Intermediate-smooth	5	8	2	15
Smooth	4	16	3	23
Total	17	31	13	61

Of the 135 F_3 lines grown from the cross Svanhals × Lion, 64 were 6-rowed and 24 were homozygous 2-rowed. In the 6-rowed group, 20 lines bred true for early heading, 11 headed late, and 33 segregated (TableXXII). In the 2-rowed group the conditions are reversed; more lines bred true for late heading than for early heading. Since the 6-rowed parent in this cross is the early parent, the factors for 6-rowed and early heading went into the cross together and tend to stay together. The linkage intensity, however, is quite low.

TABLE XXII.—*Summation of the 6-rowed and 2-rowed F_3 families with respect to the characters early heading and late heading; cross Svanhals × Lion, 1923*

	Six-rowed families			
	VV EE	VV Ee	VV ee	Total
Observed	20	33	11	64
Calculated 42 per cent C. O	21.5	31.2	11.3	64

	Two-rowed families			
	vv EE	vv Ee	vv ee	Total
Observed	4	12	8	24
Calculated 42 per cent C. O	4.2	11.9	8.1	24.2

χ^2 = less than 1. P = Good fit.

In calculating the linkage intensity, genotypes are assumed for the groups as follows:

Group	F_2 genotype	F_3 breeding behavior
a	VV EE	Breeding true for early heading and 6-rowed.
b	VV Ee	Segregating for date of heading, breeding true for 6-rowed.
c	VV ee	Breeding true for late heading and 6-rowed.
c	vv EE	Breeding true for early heading and 2-rowed.
b	vv Ee	Segregating for date of heading, breeding true for 2-rowed.
a	vv ee	Breeding true for late heading and 2-rowed.

In independent inheritance, the groups *a*, *b*, and *c* are to each other as 1:2:1 or $\sqrt{\frac{a}{c}}:1$ equals 1:1, $\frac{2a}{b}:1$ equals 1:1, and $\frac{b}{2c}:1$ equals 1:1. If one combines these three terms, the formula becomes

$$\left[\left(\sqrt{\frac{a}{c}}+\frac{2a}{b}+\frac{b}{2c}\right)\Big/3\right]:1.$$

This formula, in independent inheritance, gives 1:1, or the ratio of the gametes VE to Ve and ve to vE. In the case of linkage, the formula in the bracket will equal the noncrossover gametes in relation to one crossover gamete when the factors V and E enter the cross from one parent and v of linkage with 42 per cent crossover the fit is unusually good, X^2 being less than unity (Table XXII).

This method of deriving gametic ratios and crossover percentages may prove of considerable value for those cases in which a selection is made in F_2 for noncorrelated characters. The

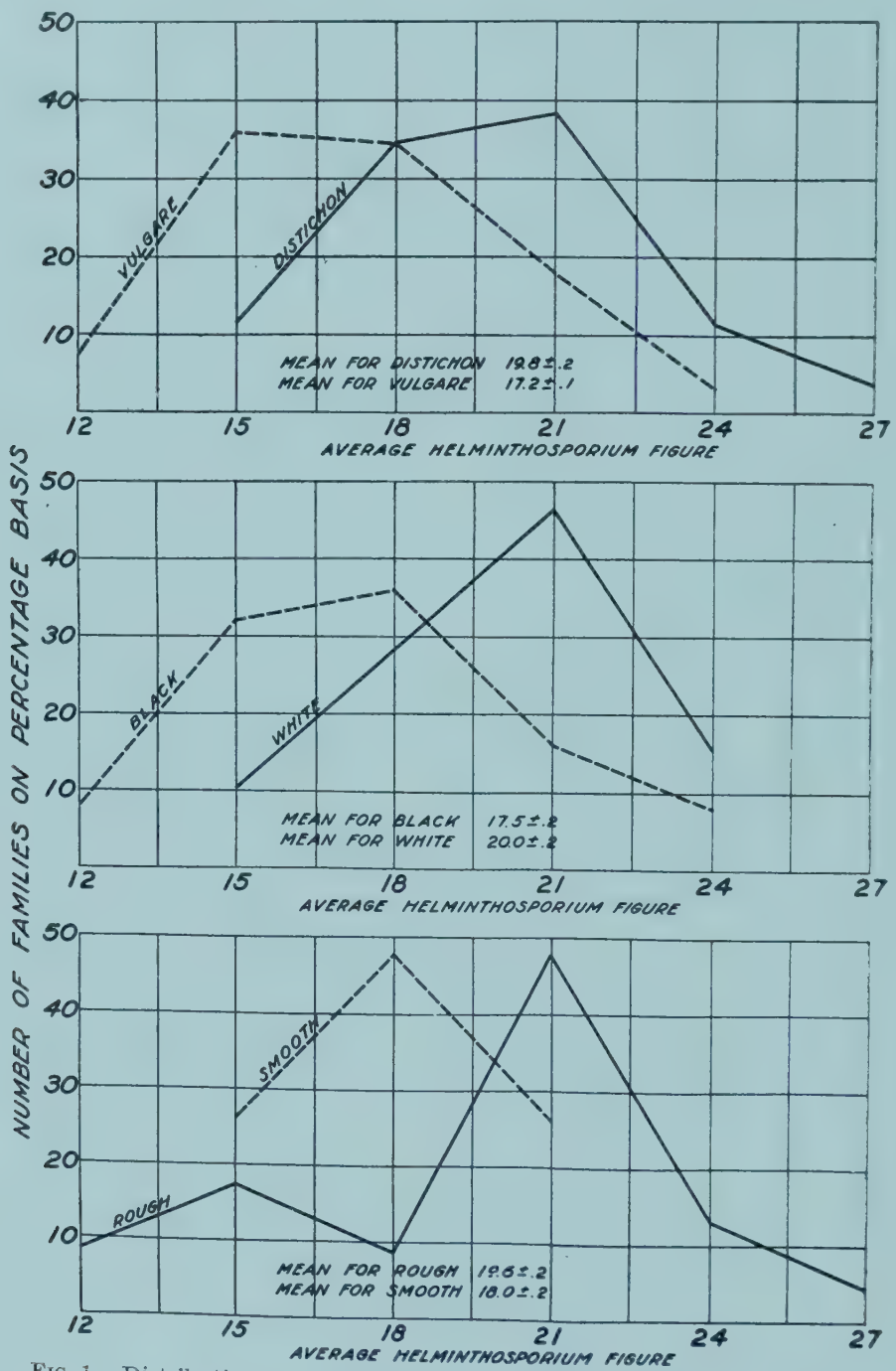


FIG. 1.—Distribution for Helminthosporium reaction of F_3 lines homozygous for other characters, cross Svanhals×Lion, 1923

and e from the other. As selection was made for the characters 2-rowed and 6-rowed and the number of families in the 6-rowed group is different from the number in the 2-rowed group, the formula should be applied to each group separately and the two results averaged. When the observed results are compared to calculated results on the basis fact that it has been valuable in this one case is considered sufficient justification for including it in this discussion.

REACTION TO HELMINTHOSPORIUM IN RELATION TO OTHER CHARACTERS.—Considerable variability is found in the reaction of a variety, even a pure line, to *Helminthosporium sativum*. For this

reason, resistance and susceptibility can not be considered as absolute characters, and it is impossible from the data obtained on an F_3 line to conclude whether or not the line is homozygous for resistance. The relation of resistance and susceptibility to other characters can be studied only by considering these groups in a comparative manner.

Keeping in mind that the factors for resistance to Helminthosporium went into the cross Svanhals × Lion with the factors for rough awn and white glumes, it is interesting to note that the white-glumed F_3 lines tend to be more resistant than the black lines and the rough-awned lines more resistant than the smooth-awned lines. (Fig. 1 and Tables XXIII and XXIV.)

TABLE XXIII.—*Relation of resistance and susceptibility to other characteristics in F^3 families of the cross Svanhals × Lion*

Distribution of F_3 families of—	Average Helminthosporium figure						Total	Average
	12	15	18	21	24	27		
2-rowed	---	3	9	10	3	1	26	19.8±0.4
6-rowed	5	24	23	12	2	---	66	17.2±0.2
Black	2	8	9	4	2	---	25	17.5±0.4
White	---	4	11	18	6	---	39	20.0±0.3
Rough	2	4	2	11	3	1	23	19.6±0.5
Smooth	---	6	11	6	---	---	23	18.0±0.3

The character pairs black versus white, 2-rowed versus 6-rowed, and rough awn versus smooth awn, were shown to be independent. Since resistance and susceptibility tend to be correlated with each of these three character pairs, it may be inferred that the chromosomes which carry the factors for the characters mentioned also carry factors for resistance and susceptibility to the spot blotch disease. Thus it may be said that at least three genetic factors are concerned in producing resistance of the type possessed by Svanhals. That resistance and susceptibility are due to definite genetic factors is shown by the fact that either may be combined with black or white, 2-rowed or 6-rowed, and rough or smooth.

TABLE XXV.—*Comparison of the Helminthosporium figure for the 6-rowed and 2-rowed F_3 lines in regard to the characters early and late*

	Average Helminthosporium figure						Total	Average figure for lines
	12	15	18	21	24	27		
6-rowed early	3	10	7	---	---	---	20	15.6±0.3
6-rowed late	---	---	2	7	1	---	10	20.7± .3
6-rowed segregating	2	11	15	5	1	---	34	17.3± .3
2-rowed early	---	---	4	---	---	---	4	18.0
2-rowed late	---	1	---	4	2	1	8	22.4±0.8
2-rowed segregating	---	2	5	4	1	---	12	19.0± .5

TABLE XXIV.—*The distribution of rough-awn and smooth-awn F_3 families in regard to Helminthosporium figure as influenced by other characters*

Families	2-rowed or 6-rowed	Black or white	Helminthosporium index						Total	Average
			12	15	18	21	24	27		
Rough-awn	6-rowed	Black	2	2	---	---	---	---	4	13.5
		White	---	2	1	2	1	---	6	19.0
	2-rowed	Black	---	---	1	7	2	1	11	21.8
		White	---	---	---	2	---	---	2	21.0
Total			2	4	2	11	3	1	23	19.6±0.5
Smooth-awn	6-rowed	Black	---	2	2	1	---	---	5	17.4
		White	---	1	3	2	---	---	6	18.5
	2-rowed	Black	---	3	5	2	---	---	10	17.7
		White	---	---	1	1	---	---	2	19.5
Total			---	6	11	6	---	---	23	18.0± .3
Intermediate smooth-awn	6-rowed	Black	---	3	2	---	---	---	5	16.2
		White	---	---	2	1	---	---	3	20.0
	2-rowed	Black	5	3	1	3	---	---	7	18.0
		White	---	---	1	3	---	---	4	20.0
Total			---	6	6	7	---	---	19	18.2± .4

Since the factor for the 6-rowed character is linked with the factor for early heading, the 6-rowed lines and the 2-rowed lines should be examined to see if a correlation exists for relation to Helminthosporium, irrespective of the characters early and late. When the average Helminthosporium figure for the 6-rowed early lines is compared with that for the 2-rowed early lines, a difference is found still to exist for Helminthosporium reaction (Table XXV). A difference is found also between the 6-rowed lates and the 2-rowed lates, likewise between the 6-rowed lines and the 2-rowed lines which are segregating for early versus late. If each of these groups is given equal weight, the figure for the 6-rowed lines is 17.9 ± 0.2[6] and for the 2-rowed lines 19.6 ± 0.4, the difference being 2 ± 0.4, which is significant when considered in the light of its probable error.

The differences between the Helminthosporium figures of the early-heading and late-heading lines is greater than the difference between the figures for the 2-rowed and 6-rowed lines. This difference may be due to a physiological resistance of lateness or to a more intense linkage between the factor for earliness with the factor for susceptibility than exists between the factor for 6-rowed and the factor for susceptibility.

In considering the degree of resistance or susceptibility in relation to other characters, one is justified in the conclusion that at least three factor pairs are concerned in the production of resistance and susceptibility. One of these factor pairs is located in the chromosome pair carrying factors for 2-rowed and 6-rowed, one in the chromosome pair carrying factors for black and white glumes, and the other in the chromosome pair carrying factors for rough awn and smooth awn.

The data given here on the relation of factors for resistance to Helminthosporium and the method of locating such factors through a study of their relation to factors for other characters were presented to the Genetics Group of the American Association for the Advancement of Science (*2*). Sax (*20*), at the same meeting, gave the results of studies in which he used a similar attack on the location of size factors. As Sax (*19*) points out, the total effect of the factor or factor group can not be estimated on account of the fact that an apparent difference in such factors may be due to differences in the intensity of their linkage with those factors with which they are associated. When, however, a sufficient number of factors have been located in a particular chromosome, it may be possible to locate a size factor definitely through studying its relation to three or more other factors.

SUMMARY

Each of the cultivated barley species, *Hordeum vulgare*, *H. intermedium*, *H. distichon*, and *H. deficiens*, has 14 somatic chromosomes. According to this, 7 linkage groups are expected.

Each of the following character pairs is shown to differ by a single genetic factor: 2-rowed versus 6-rowed, *deficiens* versus 6-rowed, black versus white glumes, early heading versus late, rough awn versus smooth, and intermediate-smooth awn versus smooth.

In the cross Svanhals × Lion the character pairs black glumes versus white, 2-rowed versus 6-rowed, rough awn versus smooth awn, and intermediate-smooth awn versus smooth were shown to be independent of each other in inheritance. Black glumes versus white glumes and *deficiens* versus 6-rowed were found to be independent character pairs in the cross *H. deficiens steudelii* × *Manchuria*.

Resistance and susceptibility to *Helminthosporium sativum* are shown to be due to definite genetic factors By studying the reaction of F_3 lines to this pathogene in relation to other characters the inference is drawn that at least three factors are concerned in the production of resistance of the type possessed by Svanhals. One factor was linked with the factor for 2-rowed, one with the factor for rough awn, and one with the factor for white glumes.

The factor for early heading was found to be linked with the factor for 6-rowed. The linkage intensity was very low, the crossover value being 42 per cent. The linkage of the factor for susceptibility to Helminthosporium with the factor for earliness is much more intense than that with the factor for 6-rowed, else earliness in itself predisposes the plant to attack by the pathogene.

From the results of the crosses discussed four linkage groups have been established.

[6] The probable error of an average of averages calculated according to the formula $E=1/N\sqrt{n_1^2e_1^2+n_2^2e_2^2 \cdot \cdot \cdot \cdot n_n^2e_n^2}$, in which n is the number of lines in a group, e the probable error of the group, and N the total number of lines considered (*17*).

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APPENDIX

TABLE XXVI.—*Distribution for date of heading in F_3 families as compared with the parent varieties; cross Svanhals × Lion, 1923*

Row No.	Date of heading									Number of plants	Classed as—	Genotype of parent plant
	18	21	24	27	30	3	6	9	12			
Lion		32	11	1			1			45	Early	EE
Do		19	16	3	1					39	do	EE
Do		23	16				1			40	do	EE
Do		18	22	1						41	do	EE
Do		32	8	3						43	do	EE
Do		21	11	4	1					38	do	EE
Do		39	2	1						42	do	EE
Do		22	6	1	6					35	do	EE
Do	1	33	9			1				44	do	EE
Do		23	9	5	4					41	do	EE
Do		26	15	2						43	do	EE
Do		23	15	4				1		43	do	EE
Do		25	13	2	2					42	do	EE
Do		20	10	2	6					38	do	EE
Svanhals						8	13	11	7	39	Late	ee
Do					1	9	20	9		39	do	ee
Do						2	16	14	5	37	do	ee
Do					2	19	19	2	1	43	do	ee
Do						6	20	5	5	36	do	ee
Do						6	17	10	4	37	do	ee
Do					1	14	25	1		41	do	ee
Do						3	19	6	6	34	do	ee
Do						15	16	7	7	45	do	ee
Do						1	20	10	4	35	do	ee
Do					1	11	22	3	2	39	do	ee
Do						3	22	9	1	35	do	ee
Do					1	15	14	7	1	38	do	ee
Do						3	25	6	3	37	do	ee
3	8	24	3	1	1		4	1		42	Segregating	Ee
4		29	14	1	2					46	Early	EE
5	1	21	7		1		3			33	Segregating	Ee
6	15	12	15							42	Early	EE
7						1	12	10	4	27	Late	ee
8							19	11	3	33	do	ee
9						1	23	6	5	35	do	ee
10	1	16	13	2			5	4	1	42	Segregating	Ee
11	23	9	5							37	Early	EE
12		17	11	2			1	4	4	39	Segregating	Ee
15		6	18	1	2	1	1	2	4	35	do	Ee
16		16	3	1	3	7	3	3		36	do	Ee
17		13	9	3	2	1	6	4	2	40	do	Ee
18		10	8	3	1	2	3	1	3	31	do	Ee
19	1	36	5	3						45	Early	EE
20	2	39	1							42	do	EE
21		18	5	2	3	3	4	3	4	42	Segregating	Ee
22		9	13	2	2		1	3	3	33	do	Ee
23						1	19	12	2	34	Late	ee
24		18	7	4			2	1	3	35	Segregating	Ee
27		33	5	2	1					41	Early	EE
28							22	12	2	36	Late	ee
29	3	20	3	1		1	2	1		31	Segregating	Ee
30						6	15	11	2	34	Late	ee
31	1	14	15	2			1	3		36	Segregating	Ee
32	1	8	15	1	1		5	5		36	do	Ee
33		35	6							41	Early	EE
34	9	28	2							39	do	EE
35		14	11	3		3	5	2	2	40	Segregating	Ee
36	4	15	6	2	1	3	6	2		39	do	Ee
39		22	12	1	1					36	Early	EE
40	2	28	4			2	1	1		38	Segregating	Ee
41	11	27	1							39	Early	EE
42	5	30	2							37	do	EE
43	1	17	10	1			6	2	1	38	Segregating	Ee
44	1	20	8	1	2		5			37	do	Ee
45						1	14	15	8	38	Late	ee
46	3	31	5							39	Early	EE
47						1	5	15	8	29	Late	ee
48	10	27	1	1						39	Early	EE
51	7	21	4		1		1	1	2	37	Segregating	Ee
52	2	20	7		1		1	1		32	Early	EE
53	4	17		1		1	11			34	Segregating	Ee
54		20	2	2		2	6	1	1	34	do	Ee
55						2	14	7	10	33	Late	ee
56	4	15	4	1			1	2		27	Segregating	Ee
57		28	5	1		2	1	1	2	40	do	Ee
58	3	17	9			3	7		1	40	do	Ee
59		24	7	1	1	3	1			37	do	Ee
60	1	32	2		1					39	Early	EE
63		15	6	4		1	4	4	2	36	Segregating	Ee
64	2	25	3	2			3			35	do	Ee
65	4	18	4	2	1	1		2		32	do	Ee

TABLE XXVI.—*Distribution for date of heading in F_3 families as compared with the parent varieties; cross Svanhals × Lion, 1923*—Continued

Row No.	Date of heading									Number of plants	Classed as—	Genotype of parent plant
	18	21	24	27	30	3	6	9	12			
66		37	3	1						41	Early	EE
67						5	20	11	4	40	Late	ee
68	2	24	3	1	1		5	3		39	Segregating	Ee
69		21	6	2		1	1	1		32	Early	EE
70		11	10	2	2	1	5	3		34	Segregating	Ee
71		15	5	1	4	2	1	1	1	30	do	Ee
72		14	6	4	1	1	1		2	29	do	Ee
75	4	19	1		2	3			1	30	do	Ee
76	1	40	1							42	Early	EE
77	4	26	3		2	1	2	1		41	Segregating	Ee
78		1				1	19	9	1	31	Late	ee
79	3	33		4						40	Early	EE
80		19	7			1	2	1	3	33	Segregating	Ee
81	3	18	4	2		2	7	1		37	do	Ee
82	1	17	10		1	1	2	4		36	do	Ee
83	3	33	4	1						41	Early	EE
84		22	6	2			2	2	3	37	Segregating	Ee
87	3	23	3		1	2	4	2	1	39	do	Ee
88	7	24	2			2	2	4		41	do	Ee
89	1	25	6			1	6	2		41	do	Ee
90					1	16	11	4	1	33	Late	ee
91					1	16	14	3	1	35	do	ee
92	16	23	2	1	1					45	Early	EE
93		23	7		1	4	4	2	1	42	Segregating	Ee
94						10	14	10	3	37	Late	ee
95						7	26	5	1	39	do	ee
96						5	22	8	2	37	do	ee
99	2	25	3	1			4		1	36	Segregating	Ee
100	2	27	3			4	2			38	do	Ee
101			1		2	5	14	10	5	37	Late	ee
102	5	21	2	2	1	4	3	1		39	Segregating	Ee
103	21	11	6							38	Early	EE
104	9	31								40	do	EE
105	12	29		1						42	do	EE
106	6	21		1		2	2	2	2	36	Segregating	Ee
107	2	27	1		2	6	2	2		42	do	Ee
108	5	31								36	Early	EE
111	18	23	2	1						44	do	EE
112	1	40	1		1					43	do	EE
113	3	25	2	4	1			1	2	38	Segregating	Ee
114						1	18	10	5	44	Late	ee
115				1	1	16	11	2	3	34	do	ee
116						1	5	15	12	33	do	ee
117	16	16	1							33	Early	EE
118						6	20	7	4	37	Late	ee
119	10	26	3		1		1			41	Early	EE
120	8	19	6		2	3	1			39	Segregating	Ee
123	11	14	4			3	3			35	do	Ee
124	5	25	4	1						35	Early	EE
125	2	25	1	2		3	6			39	Segregating	Ee
126					2	15	17	3	1	38	Late	ee
127	2	25		1			4	7	1	40	Segregating	Ee
128						12	20	6	2	40	Late	ee
129	4	15	1	1	1	1	6	3	2	34	Segregating	Ee
130	5	20	3	3		5	1			37	do	Ee
131							25	10	2	37	Late	ee
132						3	22	8	3	36	do	ee
135					2	13	15	8	1	39	do	ee
136	7	23	1	2			2	2		37	Segregating	Ee
137	2	22	1	1	1	4	6	3		40	do	Ee
138	12	14	1	1	2	7	4	1		42	do	Ee
139	16	18	1	2	3	2		2		44	do	Ee
140	3	24	2			1	1		1	32	Early	EE
141						6	19	8	1	34	Late	ee
142	2	31	1							34	Early	EE
143		14	6	1	4	1	4	4		34	Segregating	Ee
144	2	26	3				4	1	1	37	do	Ee
147	4	23	4			3	4			38	do	Ee
148		25	7				3	3	2	40	do	Ee
149	6	15	1			1	3	1		27	do	Ee
150		20	6		2			1	2	31	do	Ee
151	1	21	6	2			3	3	2	38	do	Ee
152		23	4	1	1	1	2			32	do	Ee
153	1	25	2			2	6	3	1	40	do	Ee
154		1				1	23	13	3	41	Late	ee
155	1	24	4				2	2	6	39	Segregating	Ee
156							15	12	6	33	Late	ee
159	3	15	5	2	1	1	4	1	2	34	Segregating	Ee
160							11	15	12	38	Late	ee
161	17	19	1							37	Early	EE
162	6	17	3	1	2	1	4	1		35	Segregating	Ee
163	1	16	7	3	2		1	1	2	33	do	Ee

THE RELATION OF SULPHUR TO ALFALFA PRODUCTION [1]

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INTRODUCTION

That sulphur is one of the essential elements for the proper growth and development of plants is a well-known fact. Being a constituent of most proteins which are closely associated with living matter, it probably is connected with physiological processes in the formation of compounds which do not contain sulphur. If this be true, it is apparent that sufficient quantities of sulphur must be supplied to plants in order to produce normal maximum growth.

In recent years the importance of sulphur in crop production has been given greater consideration than formerly. Probably this is due to the early and erroneous methods of analysis which indicated that crops removed very small amounts of this element from the soil. Hence it was thought that if crop residues were returned, most soils contained enough sulphur to produce maximum yields indefinitely. However, improved methods of analysis have shown that considerably more sulphur is removed by plants than was formerly thought to be the case and that in some instances it may be the limiting factor in crop production.

In field experiments sulphur deficiency has been observed most frequently with legumes. Since alfalfa is one of the most important legumes, it was deemed desirable to investigate the possibility of increasing the yields of this crop by sulphur fertilization. This paper reports 10 results of a single experiment conducted for one season.

REVIEW OF LITERATURE

Reimer and Tartar (*13*) [3] in Oregon found that the yields of alfalfa and red clover can be greatly increased by the use of sulphur-containing fertilizers. The soils on which these experiments were conducted contained only limited amounts of sulphur. Superphosphate and gypsum were found to increase the yield of alfalfa and produced a darker green color, while rock phosphate had little or no effect whatever. Other sulphur-containing fertilizers increased the yields from 50 to 1,000 per cent, larger increases being obtained in some cases than in others where the same amount of sulphur was applied. These differences were attributed to differences in depth and physical condition of the soil. The application of fertilizers containing nitrogen, phosphorus, and potassium had little or no effect on alfalfa. The same was true regarding the application of lime. This was taken to indicate that any benefits derived from sulphur-containing fertilizers could not be due to their effect upon liberating phosphorus, potassium, or lime in the soil, or upon nitrification. The root system and the number of nodules was also materially increased by the addition of sulphur. Plants receiving sulphur contained more nitrogen and more protein than those from the control plots.

Fellers (*5*) secured a material increase in the yield of total dry matter and seed of soy beans by small applications of acid phosphate on well limed soils. Small applications seemed to be as effective as larger amounts. It was observed also that acid phosphate applied on limed soil increased the nodule development and content of oil. Protein formation was benefited on both limed and unlimed soils. Land plaster produced but little effect unless applied in large amounts, in which case the oil content of the seed and the growth of nodules were stimulated. Applications of elemental sulphur in small amounts, not over 100 pounds

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[3] Reference is made by number (italic) to "Literature cited," p. 947.

per acre, produced an increase in yield, but larger amounts seemed to injure the plants. The protein content was increased by moderate applications of sulphur and decreased by large amounts. Duley (*2*) has also shown that gypsum and sulphur greatly increase nodule formation on red clover in certain Missouri soils.

Hall (*6*) found that the percentage of total sulphur in alfalfa hay from various parts of the United States varied to a considerable degree and that the fields producing the heaviest yields contained the highest percentages of sulphur. The amount of sulphur removed by average crops was found to be from 37 to 90 pounds per acre, which seems to be in excess of the amount returned to the soil by rain.

Eaton (*3*) pointed out that most of the sulphur in the soil is in the organic form and that there is a general correlation between the sulphur and organic matter content, soils high in organic matter having in general a high sulphur content. It was found that the application of large amounts of gypsum to red clover produced plants containing more nitrates, protein, and sulphates than where small amounts were added. Also flowers of sulphur and sodium sulphate containing the same amount of sulphur as 100 pounds of gypsum per acre and gypsum at the rate of 500 pounds per acre greatly increased the yield of sweet corn. Larger applications of flowers of sulphur and sodium sulphate gave no increase.

In regard to beans and peas, Hart and Tottingham (*7*) found that sulphates were beneficial in the production of seed and of hay. Calcium sulphate was found to be superior to sodium sulphate; however, both greatly increased root development and likewise produced more hay. Elemental sulphur depressed the development of the legumes studied except for an apparent increase in root development in clover. Miller (*10*) found an increase in the nitrogen content and root development of clover by the application of sulphates.

Shedd (*15*) secured an increased yield of soy beans in almost every test from the application of sulphates or sulphur. Sulphatic fertilizers increased the sulphur content of soy beans, but not necessarily the protein content, as would be expected, since sulphur is a constituent of protein. In a number of cases the per cent of protein increased as the sulphur content increased, but not in the same proportion. Ammonium sulphate was found to increase the protein content of soy beans to a marked degree.

Olson and St. John (*11*) found that alfalfa treated with sulphur-containing fertilizers produced two or more times the quantities of hay on certain Oregon soils than was obtained on land untreated. Alfalfa treated with sulphur contained more protein than where no sulphur was applied and the plants had a darker green color which was evident in the cured hay.

Pitz (*12*) concluded that calcium sulphate greatly increased root development in clover grown in artificial media, but concentrations as high as 0.1 per cent retarded root growth. Elemental sulphur gave a slight increase in yield of red clover on Miami silt loam, but there was no difference in size or number of nodules on the roots. Large amounts of elemental sulphur decreased the total number of bacteria growing on agar plates, but produced an increase in ammonification with concentrations of 0.05 per cent. This was accompanied by a decrease in nitrate formation, which was thought to be due to the acidity produced.

Erdman (*4*) obtained an increased yield of alfalfa hay at each cutting with gypsum, but the effect was more pronounced on the first cutting than on subsequent cuttings. Apparently 200-pound applications were better than 500-pound applications. Gypsum did not affect the nitrogen content of the hay. He also found that gypsum was favorable to red clover in several instances and that it had practically no effect on the crude protein content with or without lime. It was found that gypsum applied in ordinary amounts had no effect upon the types of bacteria instrumental in breaking down organic matter nor was there any apparent effect upon the ammonifiers or nitrifiers. This is practically in accord with results reported by Lipman (*9*) who states that from 200 to 500 pounds of sulphur per acre did not materially increase the lime requirement, but heavier applications did increase the lime requirement.

PLAN OF INVESTIGATION

In order to determine the effect of sulphur on alfalfa production a greenhouse experiment was planned consisting of 12 treatments of five pots each. Each pot contained 12.24 kg. of the surface 7 inches of Oswego silt loam, a black, productive residual soil derived from weathered shale. Soil samples were taken at the beginning of the experiment for the purpose of

determining the content of moisture, nitrogen, phosphorus, and sulphur. The method of treatment for the various series was as follows:

No. 1, acid phosphate 400 lbs. per acre.
No. 2, acid phosphate 400 lbs. and sulphur 384 lbs. per acre.
No. 3, lime as calcium oxide 4,000 lbs. per acre.
No. 4, sulphur 384 lbs. and lime 4,000 lbs. per acre.
No. 5, sulphur 384 lbs. per acre.
No. 6, 542 lbs. of N–P–K as 2–12–2, and lime 4,000 lbs. per acre.
No. 7, 542 lbs. of N–P–K as 2–12–2, 384 lbs. of sulphur and 4,000 lbs. of lime.
No. 8, manure 3,000 lbs. per acre.
No. 9, manure 3,000 lbs. and sulphur 384 lbs. per acre.
No. 10, manure 3,000 lbs., sulphur 384 lbs., and lime 4,000 lbs. per acre.
No. 11, control.
No. 12, inoculated sulphur 384 lbs. per acre.

The sources of fertilizing elements in the complete fertilizer were nitrogen from $NaNO_3$, phosphorus from $CaH_4(PO_4)_2$, and potassium from KCl. The different materials were added to the pots and thoroughly incorporated with the soil before bringing the pots up to the optimum water condition. The alfalfa was then planted, November 22, 1922. Because of a delay in getting a supply of inoculated sulphur, series No. 12 was not seeded until December 6, 1922. Each pot was weighed twice per week and enough distilled water added to bring it up to weight. When the plants were about half grown they became infested with red spider. The only effective way of controlling this pest is a rather strong spray of cold water from a hose. The spraying has to be done every day the sun shines and, as a consequence, some water other than distilled water was applied in this way. The plants were allowed to grow until the majority were in full bloom when they were harvested, May 8, 1923. After recording the green weights, the samples were placed in a steam drying oven and the oven-dry weights determined.

The method used in determining the content of nitrogen, phosphorus, and potassium was the same as stated in the manual of the Association of Official Agricultural Chemists, revised in 1919 (*1*). The magnesium nitrate method, as described by Swanson and Latshaw (*17*) was used in determining the sulphur content of the soil. The magnesium nitrate method, as described by Latshaw (*8*), was used in determining the sulphur in the roots and tops of the plants. The colorimetric method, as given by Schreiner and Failyer (*14, p. 49*), was used in determining the soluble sulphates of the soil.

EXPERIMENTAL RESULTS

The composition of the Oswego silt loam at the beginning of the experiment is as follows: Surface soil—total moisture, 19.31 per cent; nitrogen, 0.228 per cent; phosphorus, 0.061 per cent; potassium, 2.119 per cent; total sulphur, 0.065 per cent; P_H value, 7.016 per cent; and soluble sulphates, 34.6 parts per million. In general these figures agree rather favorably with results of Swanson and Miller (*16*) but the potassium and sulphur content is somewhat higher than that reported by these investigators.

The mechanical analysis of Oswego silt loam as reported by the Bureau of Soils, United States Department of Agriculture, is as follows: Surface soil—fine gravel, 0.0 per cent; coarse sand, 0.6 per cent; medium sand, 0.3 per cent; fine sand, 1.5 per cent; very fine sand, 6.9 per cent; silt, 71.8 per cent; and clay, 17.8 per cent.

The daily minimum and maximum temperatures for the period of the experiment are shown in Table I. No records for the week ending December 30, 1922, are available.

The yields for the first and second cuttings of each treatment are given in Table II, in which it will be observed that the treatments which have given the greatest returns are lime, sulphur lime, and N–P–K with sulphur and lime (pl. 1). The difference in mean yield between the series named above in the respective order and the control is 2.0, 3.0, and 3.9 gm. per pot. These treatments, however, do not give distinctly significant increases when the probable error calculated by Bessel's formula is considered.

The weight of roots for the various treatments are given in Table III. The probable errors calculated by Bessell's formula are given for the yields but the numbers in which they are based are small and probably they should be used with caution in interpreting the results.

Acid phosphate and sulphur, sulphur, N–P–K with lime, manure, and inoculated sulphur are treatments resulting in decreases in yield of alfalfa (pl. 2). Here again, the decreases are not significant on the basis of the probable error as calculated by Bessel's formula.

Considering root growth, as shown in Table III, there are not any significant increases in the effect of the various treatments, but three significant decreases: Acid phosphate;

manure and sulphur; manure, sulphur, and lime, all produced decreases in root development greater than three times the probable error of the difference. A careful examination of the roots was made for nodules but no differences in the size or number corresponding to differences in treatment were observed.

Plates 1 and 2 portray the differences in top growth for the first and second cuttings. For the first cutting there was a noticeably better growth of alfalfa in the limed and sulphur-with-lime treatments. This difference was not borne out in the second cutting, all treatments producing approximately the same growth response.

TABLE I.—*Minimum and maximum daily temperature*

Week ending—	Sunday		Monday		Tuesday		Wednesday		Thursday		Friday		Saturday	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.
Nov. 25	59	77	64	73	63	80	58	85	58	80	51	77	52	91
Dec. 2	53	83	56	83	60	78	62	87	52	82	57	90	55	80
Dec. 9	50	73	58	81	49	85	60	76	57	73	57	77	53	76
Dec. 16	57	72	59	72	56	88	50	77	58	78	58	81	61	78
Dec. 23	57	81	61	90	61	82	65	87	60	82	63	81	63	85
Jan. 6	60	93	58	78	50	81	60	85	74	83	60	83	63	84
Jan. 13	65	94	67	78	65	81	68	86	65	83	67	86	60	83
Jan. 20	64	80	54	86	66	87	69	82	60	76	66	82	62	88
Jan. 26	56	80	62	85	62	78	63	85	67	81	61	82	69	86
Feb. 2	71	87	68	88	68	76	63	73	64	91	66	88	61	88
Feb. 10	70	95	66	86	65	87	65	86	69	88	58	85	58	80
Feb. 17	70	93	69	90	57	86	64	80	64	88	63	86	60	83
Feb. 24	74	87	68	81	65	85	69	90	55	88	67	90	71	88
Mar. 3	70	92	64	87	63	95	59	84	70	95	70	92	57	85
Mar. 10	65	86	65	81	60	95	65	88	63	90	62	92	70	92
Mar. 17	67	76	65	82	61	95	61	77	68	80	63	92	65	92
Mar. 24	52	88	66	85	71	88	62	95	62	80	63	88	65	97
Mar. 31	61	88	60	95	61	92	65	90	61	95	60	75	66	95
Apr. 7	57	90	67	78	63	80	67	72	68	80	70	81	61	81
Apr. 14	72	85	66	77	65	80	67	81	68	78	68	85	63	76
Apr. 21	65	81	63	82	70	86	68	85	75	83	68	77	65	79
Apr. 28	65	75	67	86	62	85	67	75	64	78	65	75	65	82
May 5	63	88	65	85	70	80	65	88	65	87	65	83	60	90
May 12	60	91	67	84	57	83	57	83	63	94	70	87	60	89

TABLE II.—*Yields of different treatments for first and second cuttings*

Pot No.	Treatment	Dry weight			Difference in yield compared with control	Ratio $\frac{D}{Ed}$
		First cutting	Second cutting	Total		
		Gm.	Gm.	Gm.	Gm.	
1	Acid phosphate	40.0	47.5	87.5		
2	do	42.5	44.5	87.0		
3	do	42.5	43.0	85.5		
4	do	36.5	42.0	78.5		
5	do	47.5	37.0	84.5		
	Average			84.6 ±1.085	−0.4±3.32	0.12
6	Acid phosphate and sulphur	43.0	45.0	88.0		
7	do	34.5	42.0	76.5		
8	do	31.0	39.5	70.5		
9	do	29.0	40.5	69.5		
10	do	36.5	40.5	77.0		
	Average			76.3 ±2.273	−8.7±3.87	2.24
11	Lime	39.5	44.5	84.0		
12	do	44.5	47.0	91.5		
13	do	46.5	46.0	92.5		
14	do	34.3	42.5	77.0		
15	do	47.5	42.5	90.0		
	Average			87.0 ±1.956	−2.0±3.70	0.54

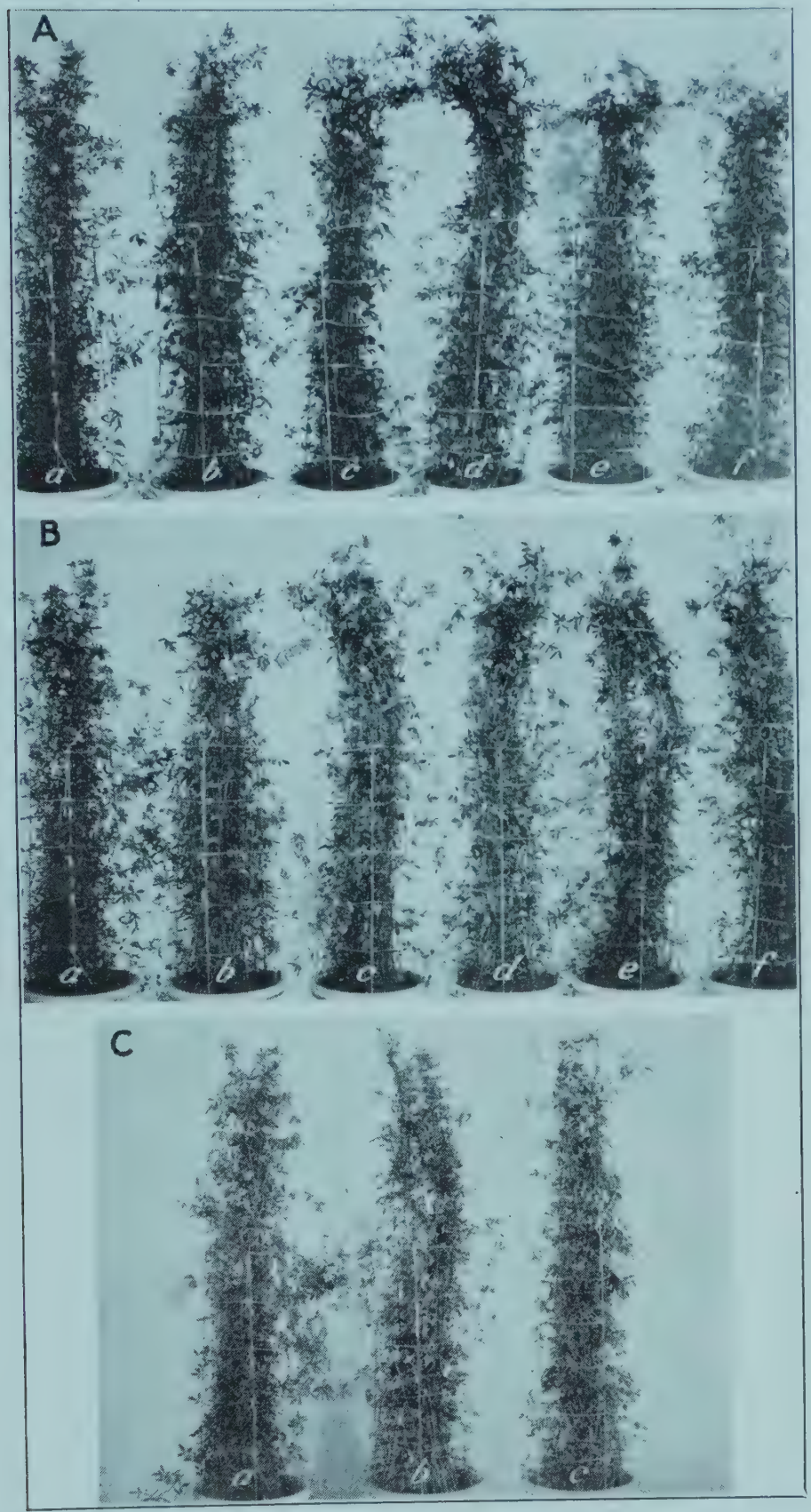
TABLE II.—*Yields of different treatments for first and second cuttings*—Continued

Pot No.	Treatment	Dry weight				Difference in yield compared with control	Ratio D Ed
		First cutting	Second cutting	Total			
		Gm.	*Gm.*	*Gm.*		*Gm.*	
16	Sulphur and lime	41.5	45.5	87.0			
17	do	39.0	46.8	85.5			
18	do	48.0	49.5	97.5			
19	do	55.5	45.0	100.5			
20	do	30.5	39.0	69.5			
	Average			88.0	±3.682	−3.0±3.68	0.81
21	Sulphur	42.5	35.5	78.0			
22	do	42.5	41.0	83.5			
23	do	34.0	41.0	75.0			
24	do	32.0	45.5	77.5			
25	do	31.5	44.5	76.0			
	Average			78.0	±0.694	−7.0±3.20	2.18
26	N–P–K and lime	36.5	47.0	83.5			
27	do	38.0	45.5	83.5			
28	do	37.0	39.5	76.5			
29	do	39.5	47.0	86.5			
30	do	32.5	47.5	80.0			
	Average			82.0	±1.36	−3.0±3.42	0.87
31	N–P–K, sulphur, and lime	39.5	54.0	93.5			
32	do	47.5	49.5	97.0			
33	do	46.5	44.5	91.0			
34	do	34.0	45.5	79.5			
35	do	38.0	45.5	83.5			
	Average			88.9	±2.178	−3.9±3.82	1.02
36	Manure	35.0	43.0	78.0			
37	do	33.5	40.0	73.5			
38	do	36.0	44.0	80.0			
39	do	34.5	44.0	78.5			
40	do	47.0	41.5	88.5			
	Average			79.7	±1.666	−5.7±2.77	2.05
41	Manure and sulphur	41.0	42.5	83.5			
42	do	48.0	46.5	94.5			
43	do	41.5	46.0	87.5			
44	do	40.5	42.5	83.0			
45	do	38.5	41.5	80.0			
	Average			85.7	±2.50	− 7±3.24	0.21
46	Manure, sulphur, lime	35.5	33.5	69.0			
47	do	34.0	43.0	77.0			
48	do	38.0	46.0	84.0			
49	do	49.5	45.0	94.5			
50	do	31.5	58.5	90.0			
	Average			82.9	±2.994	−2.1±4.22	0.49
51	No treatment	35.0	33.5	68.5			
52	do	36.5	43.0	79.5			
53	do	38.0	46.0	84.0			
54	do	33.5	45.0	78.5			
55	do	36.0	58.5	94.5			
	Average			81.0	±3.143		
56	Inoculated sulphur	32.5	39.5	72.0			
57	do	34.5	40.0	74.5			
58	do	33.5	39.5	73.0			
59	do	38.5	42.5	81.0			
60	do	38.0	42.5	80.5			
	Average			76.2	±1.281	−8.8±3.38	2.60

A.—First cutting of alfalfa showing growth of plants under various treatments; *a*, Control; *b*, Acid phosphate; *c*, Sulphur; *d*, Lime; *e*, Sulphur and lime; *f*, Manure

B.—First cutting of alfalfa, showing character of growth; *a*, Control; *b*, N-P-K plus lime; *c*, N P-K plus lime and sulphur; *d*, Manure and sulphur; *e*, Manure plus sulphur and lime; *f*, Acid phosphate and sulphur

C.—First cutting of alfalfa showing the growth of plants; *a*, Control; *b*, Inoculated sulphur; *c*, Sulphur



A.—Second cutting of alfalfa at time of harvest; *a*, Control; *b*, Acid phosphate; *c*, Sulphur; *d*, Lime; *e*, Sulphur and lime; *f*, Manure

B.—Second cutting of alfalfa; *a*, Control; *b*, N–P–K plus lime; *c*, N–P–K plus lime and sulphur; *d*, Manure and sulphur; *e*, Manure plus sulphur and lime; *f*, Acid phosphate plus sulphur

C.—Second cutting of alfalfa showing growth of plants; *a*, Control; *b*, Inoculated sulphur; *c*, Sulphur

TABLE III.—*Weight of roots for various treatments*

Pot No.	Treatment	Dry weight	Difference compared with control	Ratio $\frac{D}{Ed}$
		Gram	*Gram*	
1	Acid phosphate	26.5		
2	do	22.0		
3	do	27.5		
4	do	24.0		
5	do	17.5		
	Average	23.5 ±1.10	−7.7±1.42	5.4
6	Acid phosphate and sulphur	32.0		
7	do	25.0		
8	do	40.0		
9	do	33.5		
10	do	37.5		
	Average	33.6±0.19	−2.4±0.87	2.7
11	Lime	29.5		
12	do	35.0		
13	do	27.0		
14	do	26.5		
15	do	25.0		
	Average	28.6±1.18	−2.6±1.45	1.7
16	Sulphur and lime	25.0		
17	do	39.0		
18	do	36.0		
19	do	23.0		
20	do	15.5		
	Average	27.7±3.07	−3.5±3.1	1.1
21	Sulphur	14.5		
22	do	27.0		
23	do	15.5		
24	do	36.0		
25	do	36.0		
	Average	25.8±2.79	−5.4±2.93	1.8
26	N-P-K and lime	28.5		
27	do	33.5		
28	do	21.0		
29	do	32.5		
30	do	27.5		
	Average	30.6±2.41	−0.6±2.55	0.2
31	N-P-K, sulphur, and lime	31.5		
32	do	27.0		
33	do	25.0		
34	do	26.5		
35	do	23.5		
	Average	26.7±0.903	−4.5±1.23	3.65
36	Manure	37.5		
37	do	38.0		
38	do	28.5		
39	do	30.0		
40	do	21.0		
	Average	31.0±2.117	−0.2±2.27	0.08
41	Manure and sulphur	24.5		
42	do	25.0		
43	do	32.5		
44	do	28.0		
45	do	16.5		
	Average	25.3±1.767	−5.9±1.95	3.02
46	Manure, sulphur, and lime	13.0		
47	do	20.5		
48	do	23.0		
49	do	26.5		
50	do	32.0		
	Average	23.0±2.077	−8.2±2.23	3.67

TABLE III.—*Weight of roots for various treatments*—Continued

Pot No.	Treatment	Dry weight	Difference compared with control	Ratio $\frac{D}{Ed}$
		Gram	*Gram*	
51	No treatment	30.5		
52	do	34.5		
53	do	30.0		
54	do	33.5		
55	do	27.5		
	Average	31.2±0.849		
56	Inoculated sulphur	28.5		
57	do	26.0		
58	do	32.5		
59	do	35.5		
60	do	25.0		
	Average	29.5±1.32	−1.7±1.57	1.08

Chemical determinations were made for the acidity of the soil, soluble sulphates in the soil, and for sulphur and nitrogen in the plants. These data are presented in Table IV with a summary of the yields and probable errors. This table shows that the nearest approach to a significant difference in the results of the various treatments is in the case of acid phosphate and sulphur and the inoculated sulphur (pl. 3). Both of these treatments caused decreases in yield.

The P_H values of the soil in the respective pots give a higher hydrogen-ion content for the inoculated sulphur, which may account for its effect in causing a decrease in yield. The soluble sulphates present in the soil are low for the acid phosphate-sulphur treatment compared with all others; however the percentage of sulphur in the tops equaled that present with other treatments except in the roots.

The nitrogen analyses would indicate that about the same amount of protein was formed with the respective treatments.

TABLE IV.—*Summarized data comparing effect of treatment with chemical determinations*

Treatment	Difference in yield compared with control. Total both cuttings	Difference necessary to be significant (3.2 × P. E.)	P_H value of soil after—		Soluble sulphates in soil	Sulphur in tops, first cutting	Sulphur in roots	Nitrogen in tops, first cutting
			First crop	Second crop				
					P. p. m.	*Per cent*	*Per cent*	*Per cent*
Acid phosphate	−0.4	10.6	7.827	7.151	40.3	0.4375	0.4237	3.13
Acid phosphate and sulphur	−8.7	12.38	7.151	6.678	21.1	.4877	.3157	3.39
Lime	−2.0	11.8	8.402	8.368	36.8	.4295	.4023	3.27
Sulphur and lime	−3.0	11.7	8.368	8.639	67.3	.4238	.3799	3.13
Sulphur	−7.0	10.2	6.881	7.084	43.2	.4361	.4244	3.18
N-P-K and lime	−3.0	10.9	8.233	7.861	45.0	.4591	.3832	3.43
N-P-K, sulphur, and lime	3.9	12.2	8.233	8.639	73.5	.4714	.4378	3.33
Manure	−5.7	8.8	5.664	6.407	46.8	.4330	.3957	3.33
Manure and sulphur	−0.7	10.3	8.997	7.151	47.8	.4359	.4372	3.10
Manure, sulphur, and lime	−2.1	13.5	8.436	8.436	49.9	.4246	.4462	3.08
No treatment			6.441	6.137	54.3	.4451	.4045	3.35
Inoculated sulphur	−8.8	10.8	4.514	4.311	76.4	.4203	.3967	3.23

An average-sized root system of alfalfa plants removed from the soil immediately after harvesting the second crop

SUMMARY

The data presented in these investigations show that the type of soil used in these experiments contained as much of the common fertilizing elements as the average Kansas soil and the sulphur content to be a little higher than the average.

Sulphur applied to alfalfa has given no marked increase in yield or root development. This means that sulphur is not the limiting factor in alfalfa production on the type of soil used in this experiment.

In general, the acidity of the soil was increased by the sulphur, evidence that it should be supplemented with lime.

The nitrogen content of the tops, and the sulphur content of roots and tops were not influenced by the sulphur applied.

There was not a material increase in root development nor any increase in number or size of nodules on the roots as a result of sulphur applications.

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CARBON TRICHLORIDE AS AN ANTHELMINTIC, AND THE RELATION OF ITS SOLUBILITY TO ANTHELMINTIC EFFICACY[1]

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INTRODUCTION

On the basis of a very careful and extensive study of the correlation of the chemical composition and anthelmintic efficacy of a number of drugs, Caius and Mhaskar (*3*)[2] arrived at the conclusion that since chloroform showed considerable anthelmintic efficacy against hookworms, some related compound with a higher halogen content might prove even more effective, the anthelmintic efficacy in the case of chloroform being correlated with its chlorine content. By a shorter and less exhaustive line of reasoning, Hall (*6*) derived the same conclusion from substantially the same premises and reported that carbon tetrachloride was such an anthelmintic, his paper appearing the same month that Caius and Mhaskar predicted the likelihood of a compound related to chloroform being effective against hookworms. Since chloroform ($CHCl_3$) and carbon tetrachloride (CCl_4) show considerable efficacy against hookworms, it seems desirable that other related compounds should be investigated. In a study of miscellaneous anthelmintics, Hall and Shillinger (*7*) have tested ethylene dichloride ($C_2H_4Cl_2$), and find that it has some efficacy against hookworms, but less than chloroform and carbon tetrachloride. Recently Hall and Shillinger (*8*) have reported that tetrachlorethylene (C_2Cl_4) is apparently even more effective than carbon tetrachloride in removing hookworms, their paper covering experiments carried out subsequent to those reported in this paper and continuing the same line of investigation. The investigation reported in this paper pertains to carbon trichloride or hexachlorethane (C_2Cl_6).

Carbon trichloride occurs in the form of colorless crystals which are insoluble in water, but soluble in alcohol, ether, carbon tetrachloride, and all oils. Its insolubility in water is a conspicuous property. When 1 gram was added to 2,000 c. c. of water there was no apparent diminution in the amount of it after two months, so little went into solution. The apparent solubility was decidedly less than 1 in 10,000. Its odor is suggestive of camphor or turpentine. On theoretical grounds, certain possibilities as regards anthelmintic action might be predicted from the chemical composition and physical nature of this substance. From its atomic structure one might conclude that it would be less effective than carbon tetrachloride, since its proportion of chlorine to carbon is 3 : 1 instead of 4 : 1. However, since the molecule actually contains more chlorine (6 atoms) than does carbon tetrachloride (4 atoms), it might prove actually more effective. Actual test throws no light on this subject of chlorine content versus chlorine concentration, for the decisive factor in connection with its internal action is the physical factor of solubility.

Anthelmintics have long been regarded as relatively insoluble, more or less toxic, substances, which, by virtue of their relative insolubility, would remain in the digestive tract to a great extent and poison the worms present, the host meanwhile absorbing very little of the poison and the drug being swept out of the digestive tract by means of a purge as soon as feasible. However, Hall (*5*) has noted as a principle of anthelmintic medication that "anthelmintics of the supposedly insoluble type are not as insoluble as they are commonly supposed to be," and cites the findings of Seidell in regard to thymol, to the effect that "of the thymol administered, from one-half to two-thirds is apparently destroyed or temporarily fixed in the body." Thymol, we may recall, is soluble about 1 part in 1,100 parts of water at 25° C. Carbon tetrachloride is soluble 1 part in 1,250 parts of water at 25° C.

As carbon tetrachloride appears to be about the least water-soluble of the anthelmintics in use for hookworms,

[1] Received for publication July 31, 1924; issued July, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 953.

its solubility in and absorption from the digestive tract are matters of some little interest in connection with our study of an even more insoluble chlorine compound. Hall (*6*) pointed out in his first paper that the safety of carbon tetrachloride appeared to be correlated with its insolubility. Chopra and McVail (*4*) say: "Owing to its low solubility and volatility and consequently slow rate of diffusion, only small quantities are absorbed into the circulation. Large quantities can therefore be introduced into the alimentary canal without untoward effects." So far as we can judge from the available evidence, the amount of absorption of carbon tetrachloride from the digestive tract does not usually increase as the amount administered is increased, or if it does, there seems to be a compensatory elimination of the larger amounts of the drug without a concomitant and strictly proportionate increase in liver injury. Wells (*11*) has recently shown that a high percentage of the absorbed carbon tetrachloride is rapidly excreted in the expired air.

The experience of various workers indicates that a certain amount of drug is absorbed usually and that it causes a moderate amount of injury to the liver, this injury being rapidly and completely repaired. In fact, Bose and Mukerji (*1*) have reported two cases in which a levulose test of liver function before and after treatment with carbon tetrachloride showed deficiency of liver function before treatment and normal function after treatment, a thing theoretically possible where a chronic indolent pathological condition is rendered acute and stimulated to repair. When large amounts of the drug, 25 c. c. per kilo, or totals of several hundred c. c. at a time, or up to 2 liters in repeated doses, are administered to dogs, as has been done, it seems clear that if the absorption and a concomitant liver injury increased proportionately these animals would be killed in all cases, as we know that animals may be killed with therapeutic doses if conditions are such as to facilitate absorption, for instance, such conditions as appear to be present in certain cases of hemorrhagic enteritis. However, normal dogs commonly survive these heavy doses, showing that absorption or the tissue injury from the absorbed drug is limited; almost all the deaths reported in man and dogs followed therapeutic doses.

Lamson, Gardner, Gustafson, Maire, McLean and Wells (*9*) report that of 35 dogs given 100 to 500 times the therapeutic dose only 1 died. To put it in other words, there is no such thing as a minimum lethal dose of carbon tetrachloride for such animals as man and dog, and the same is true for chickens. Individuals of high individual intolerance for the drug may be killed with therapeutic doses, but normal individuals will tolerate enormous doses, and the limit of tolerance for normal dogs appears to be approximately the limit of gastric capacity. Chloroform, a much more soluble product, soluble 1 part in 161 parts of water at 22° C., is apparently much more readily and regularly absorbed in the digestive tract than is carbon tetrachloride, or a resultant injury is more regularly produced. Caius and Mhaskar (*2*) state in regard to chloroform: "Toxic symptoms are occasionally noticed with a dose of 30 minims [2 c. c.] and become more general and more marked with increasing dosages until 45 minims [3 c. c.] is reached." A dose of 3 c. c. of carbon tetrachloride is the one most used for human adults and usually causes no symptoms or only slight and transient symptoms. Much larger doses have been administered without evident bad effects. Peyre (*10*) has reported no inconvenience from carbon tetrachloride in a case of hepatic cirrhosis with ascites, precisely the sort of case in which bad results would follow if absorption and proportionate injury occurred to any marked extent.

Reasoning from the increased efficacy and safety of carbon tetrachloride as compared with chloroform, and associating it with its lesser water-solubility as perhaps the determining factor for these qualities, the writers have raised the question as to whether a halogen compound less soluble than carbon tetrachloride might be still more effective against hookworms, or safer, or more effective and safer. Carbon trichloride is evidently a less soluble halogen compound. The following experiments were made to test it.

EXPERIMENTAL DATA

COARSE CRYSTALS ADMINISTERED IN CAPSULES

Six dogs were given the coarse crystals in capsules, the number of each dog, its weight, its total dose, and its dose rate in terms of grams per kilo (g. p. k.) being as follows: Dog. No. 665, 13 kilos, 1 gm. (0.077 g. p. k.); No. 666, 10 kilos, 2 gm. (0.2 g. p. k.); No. 667, 6.5 kilos, 3 gm. (0.46 g. p. k.);

No. 668, 9.5 kilos, 4 gm. (0.42 g. p. k.); No. 669, 7 kilos, 1 gm. (0.14 g. p. k.); No. 670, 6 kilos, 6 gm. (1 g. p. k.). These dogs had been fasted overnight previous to the morning of treatment. The feces next day showed no worms, except 3 gravid Dipylidium segments, but all of the feces contained an abundance of the carbon trichloride crystals, the amount seen being such as to suggest that the total amount passed was practically identical with the amount administered. The crystals seemed unchanged in size, shape, and general appearance. The following day there were no worms in the feces except Dipylidium segments, and there were no crystals. Satisfied that carbon trichloride crystals alone were approximately as insoluble in the digestive tract as in water, that they passed through the digestive tract, for the most part, in the course of 24 hours, and that they did not remove worms, the theory that this oil-soluble chemical might be put in contact with worms and its anthelmintic constituent rendered available and effective by the simultaneous administration of castor oil was next tested as follows.

FINE POWDER ADMINISTERED IN CAPSULES WITH CASTOR OIL

Crystals of carbon trichloride were finely powdered in a mortar and administered in capsules, this dose being immediately preceded or followed by 1 ounce of castor oil. The number of each dog, its weight, and its total dose were as follows: Dog No. 680, 7 kilos, 5 gm.; No. 679, 7 kilos, 6 gm.; No. 678, 8 kilos, 7 gm.; No. 683, 10.5 kilos, 8 gm.; No. 682, 9 kilos, 9 gm.; No. 681, 9 kilos, 10 gm. The following day the finely powdered carbon trichloride was visible in the feces. Dog No. 679 passed 1 whipworm the next day and 2 whipworms the day after, a total of 3 whipworms, and No. 680 passed a hookworm the day after treatment. No other worms were passed the fourth day, and on that day the dogs were killed and examined postmortem. The worms present postmortem were as follows: Dog No. 680, 35 hookworms; No. 679, 24 whipworms; No. 678, 5 hookworms, 16 whipworms; No. 683, 25 hookworms, 75 whipworms, 1 Dipylidium; No. 682, 1 hookworm and 1 whipworm; No. 681, negative. Dog 679 passed 3 whipworms and had 24 left; there were 92 whipworms left in the other dogs. Dog 680 passed 1 hookworm and had 35 left; there were 31 hookworms left in other dogs. This experiment suggests that the finely powdered carbon trichloride in the presence of castor oil may become slightly effective against worms, but it indicates that the chemical would probably never be of value as an anthelmintic. To give this feature of oil solubility a more definite test, we tried the carbon trichloride in oil as follows.

CARBON TRICHLORIDE DISSOLVED IN CASTOR OIL

One gram of carbon trichloride was dissolved in 100 c. c. of castor oil with the aid of moderate heat, the solution becoming a brownish amber color. The entire amount was given by drench to dog No. 669. This animal passed no worms the next day, passed 7 whipworms the next day, none the next 2 days, and on post-mortem examination the fourth day after treatment had 14 hookworms, 18 whipworms and 40 to 50 tapeworm heads. The treatment appears to have removed 28 per cent of the whipworms. While little can be concluded from the foregoing experiments as to the effects of oil on the availability of the anthelmintic constituents of the chemical, the results suggest that this highly insoluble drug is entirely ineffective and unavailable when given alone as coarse crystals; that it becomes slightly effective and available when given as a fine powder accompanied by castor oil; and that it becomes still more effective, though by no means really valuable, when it is dissolved in oil and then administered.

Since the efficacy of oil of chenopodium for removing worms from dogs is fairly well known from large numbers of critical experiments, the writers next undertook to test the efficacy of carbon trichloride dissolved in this oil. The oil alone falls distinctly short of 100 per cent efficacy against hookworms in dogs, so that any marked increase in efficacy on the part of the mixture might be detected and would indicate an anthelmintic action on the part of the carbon trichloride.

CARBON TRICHLORIDE DISSOLVED IN OIL OF CHENOPODIUM

Six grams of carbon trichloride were dissolved in 20 c. c. of oil of chenopodium, without heat, and the dose rate for chenopodium for removing hookworms was used for the mixture in treating 6 dogs. This dose rate is 2 c. c. for dogs of average size or larger, and 1 c. c. for small dogs. Two c. c. was given to dogs No. 672 (14 k.) and 673 (12 k.), and 1 c. c. was given to

dogs No. 674 (6 k.), 675 (6.5 k.), 676 (6.5 k.), and 677 (6.5 k.). One ounce of castor oil was given at the same time. The worms passed during the next 4 days, those present post-mortem, and the percentage of efficacy were as follows:

Dog 672 passed 7 hookworms and had 3 post-mortem (70 per cent), passed 27 whipworms and had 1 post-mortem (96 per cent), passed 0 Dipylidium and had 17 post-mortem (0 per cent).

Dog 673 passed 0 hookworms and had 2 post-mortem (0 per cent), passed 2 whipworms and had 26 post-mortem (7 per cent), and passed 0 Dipylidium and had 8 post-mortem (0 per cent).

Dog 674 passed 0 worms and had 1 hookworm, 17 whipworms and 1 Dipylidium sp., an efficacy of 0 per cent for hookworms, whipworms, and Dipylidium.

Dog 675 passed 1 ascarid and had 0 post-mortem (100 per cent), passed 1 hookworm and had 1 post-mortem (50 per cent), and passed 1 whipworm and had 0 post-mortem (100 per cent).

Dog 676 passed 0 worms and had 4 whipworms post-mortem; efficacy 0 per cent for whipworms.

Dog 677 passed 1 hookworm and had 0 post-mortem (100 per cent); passed 0 whipworms and had 101 post-mortem (0 per cent).

The treatment removed 100 per cent of the ascarids present, 56 per cent of the hookworms present, 1.7 per cent of the whipworms present, and 0 per cent of the Dipylidium present. This represents no increase in anthelmintic efficacy over chenopodium alone. Consequently it appears that the solution in oil of an anthelmintic highly insoluble in water does little in the way of rendering the dissolved chemical available as an anthelmintic.

CARBON TRICHLORIDE DISSOLVED IN CARBON TETRACHLORIDE

As a final test carbon trichloride dissolved in carbon tetrachloride was administered at the rate of 25 gm. of the former in 100 c. c. of the latter. The protocols were as follows:

Dog 665: 0.3 c. c. per kilo of solution in capsules; passed 2 hookworms, 10 ascarids, and 16 whipworms; post-mortem, 4 whipworms; efficacy, 100 per cent for hookworms and ascarids, and 80 per cent for whipworms.

Dog 666: 0.3 c. c. per kilo in capsules; passed 1 hookworm and 4 whipworms; post-mortem 5 whipworms; efficacy, 100 per cent for hookworms and 44 per cent for whipworms.

Dog 667: 0.5 c. c. per kilo by capsule; passed 1 whipworm; post-mortem, 0 worms; efficacy, 100 per cent for whipworms.

Dog 668: 1 c. c. per kilo by stomach tube; passed 2 hookworms and 15 whipworms; post-mortem, 0 worms; efficacy, 100 per cent for hookworms and whipworms.

Dog 670: 2 c. c. per kilo by stomach tube; passed 20 hookworms and 4 whipworms; post-mortem, 0 worms; efficacy, 100 per cent for hookworms and whipworms.

The solution of carbon trichloride in carbon tetrachloride was 100 per cent effective against ascarids in the 1 case involved, 100 per cent effective against hookworms in the 4 cases involved, and 100, 100, 80, and 44 per cent effective against whipworms in the 4 cases involved. At the dose rate, which is equivalent to the therapeutic dose rate for carbon tetrachloride (0.3 c. c. per kilo), the solution removed all the ascarids and hookworms and 44 and 80 per cent of the whipworms. This is just about what would be expected from the carbon tetrachloride alone. In the higher rates the efficacy against whipworms rises, as it should theoretically, and as it does frequently in actual practice. Apparently the addition of the carbon trichloride to the carbon tetrachloride does not add to or detract from the anthelmintic efficacy of the latter chemical.

PATHOLOGY

In order to obtain additional information, if possible, in regard to the solubility of carbon trichloride and also its possible toxic effects, portions of the liver and kidneys of dogs Nos. 665, 666, 667, 668, and 670 (receiving carbon-trichloride crystals and afterwards carbon trichloride in carbon tetrachloride), and of No. 669 (receiving carbon-trichloride crystals and afterwards carbon trichloride dissolved in castor oil), were submitted to the Pathological Division, Bureau of Animal Industry, for examination. These tissues were examined by G. T. Creech, who reported that he found in all of them lesions similar to those which have been described by various writers as following the administration of carbon tetrachloride. In the livers there was more or less capillary congestion and the cells showed alterations varying from cloudy swelling to complete degeneration and atrophy of the cells. The kidneys showed hemorrhages, especially in the cortical por-

tions, and the glomeruli and tubular epithelium showed distinct alterations. As the kidneys of dogs commonly show pathologic conditions, little can be concluded from the findings here. That changes along these lines should occur in the case of dog No. 669, which was given carbon trichloride alone, suggests that the chemical had been absorbed to some extent in spite of its great insolubility, but as only one animal was examined in this connection not very much can safely be concluded from this case. It does establish the presumption that some carbon trichloride is absorbed and that it may affect the liver unfavorably.

SUMMARY

Experiments with carbon trichloride administered as coarse crystals in capsules, or as a fine powder in capsules followed or preceded by castor oil, or dissolved in castor oil, or dissolved in oil of chenopodium, or dissolved in carbon tetrachloride, all indicate that carbon trichloride has no value as an anthelmintic. Since the high chlorine content of carbon trichloride would indicate, on theoretical grounds, that this chemical would be quite effective in removing hookworms, the failure of the chemical to display such efficacy in actual practice is correlated with the great actual and relative insolubility of the chemical, the lack of solubility maintaining the anthelmintic chlorine constituent in an unavailable condition.

Considering the solubilities and efficacies of four chlorine compounds of the ethane and methane series, arranged in the order of anthelmintic efficacy, with the most effective first, they are as follows: CCl_4, $CHCl_3$, $C_2H_4Cl_2$, and CCl_3. Their respective water solubilities are: 1 in 1,250; 1 in 161; 1 in 120; 1 in over 10,000. The peak of anthelmintic efficacy is reached at a solubility of 1 : 1,250 (carbon tetrachloride). As the curve of anthelmintic efficacy falls away on the side of increasing solubility, the efficacy decreasing as the solubility increases, it does not appear to promise anything of value in the way of anthelmintic discovery to investigate chlorine compounds of these series which are more soluble than 1 : 1,250, although such investigations have considerable theoretical interest. The anthelmintic efficacy falls to practically zero on the side of decreased solubility for the highly insoluble carbon trichloride. There is still the possibility that a chlorine compound of approximately the same chlorine content as carbon tetrachloride, but slightly less soluble, might be safer if not more effective. Tetrachlorethylene may be such a drug. Evidently carbon trichloride is too far in the direction of insolubility. Judging from these experiments, carbon trichloride is so insoluble that its theoretically effective chlorine content is unavailable and ineffective.

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A STUDY OF THE INFLUENCE OF INOCULATION UPON THE FERMENTATION OF SAUERKRAUT [1]

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INTRODUCTION

The older literature on the subject of sauerkraut fermentation is concerned chiefly with a study of the microorganisms found in kraut without devoting much attention to the kinds and amounts of fermentation products. A review of this literature has been given in a previous publication [2] and need not be repeated here. In papers [3] published from these laboratories the chief fermentation products contained in sauerkraut were found to be: Lactic acid, 0.93 to 1.65 per cent; acetic acid, 0.22 to 0.47 per cent; ethyl alcohol, 0.14 to 0.84 per cent. The variation in composition depends upon many factors such as the age of the kraut, the type of organisms present, and the temperature and completeness of the fermentation.

The success which has attended the use of selected cultures of bacteria in the butter and cheese industry and in the growing of nitrogen-assimilating legumes suggests the use of similar methods in the kraut industry.

Some data were submitted in a previous paper [4] to show that the quality of the kraut and the quantity of the fermentation products could be influenced by the use of selected cultures of lactic acid bacteria. This work has been continued on a larger scale for the past two years and the results obtained are presented in this paper.

EXPERIMENTAL DATA

It was planned to study the chief bacteriological and chemical changes which take place at different times during the fermentation in both an uninoculated and inoculated kraut. The organism selected for the inoculation of the kraut was a strain of *Streptococcus lactis* originally isolated from milk. The use of this culture had consistently given a better quality of kraut than any of the other organisms tried and also better than the uninoculated control.

The shape of the organism, the formation of a smooth firm curd in milk, and its failure to ferment xylose increased the possibility of its detection in kraut. Its chemical products are also distinctive. It has been shown that this strain of *Streptococcus lactis* ferments the common sugars with the formation of active lactic acid and the production of little or no carbon dioxide, alcohol, or acetic acid.

Although some of the results previously reported were obtained on kraut made under factory conditions, much of it was made in large percolators of 3-liter or 4-liter capacity or small 2-gallon stone jars. In order to simulate factory conditions more closely, two large cypress vats about 5 feet high and 2 feet in diameter were constructed. A small well was built on the inside of each vat. This was made by attaching a V-shaped trough, made from two 3-inch boards, to the inside of the vat, so that the brine could rise freely in it. Samples of brine could be taken from these wells at any time during the fermentation period without distrubing the contents of the vat. It was found that as the fermentation proceeded the acidity of the brine in the wells was not the same as that of the brine in the vat, so a hole was bored through the side of the vat about 2 feet from the bottom and samples of the brine were drawn from here through a glass tube inserted through a rubber stopper. In order to obtain the temperature of the brine, a theremometer was suspended deep in the well.

The vats were filled with 250 pounds of cut cabbage at a kraut factory near Madison, Wis. The cabbage used for filling the vats was the same as that used in the factory and was prepared

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in the same manner. While the cabbage was being placed in the vats, 2½ per cent of common salt was added by the regular salter of the factory.

The contents of one vat were inoculated with about 2 liters of a three-day-old culture of lactic acid bacteria in a 1 per cent glucose-yeast-water medium. This culture was diluted with an equal volume of water and applied to the cabbage with a small water sprinkler while the cabbage was being placed in the vat. An equal amount of water was added to the control which received no inoculation.

When filled, the vats were shipped to the laboratory and stored in a room where the temperature was approximately 20° C. Analyses were begun about 24 hours after the cabbage was packed into the vats. Whenever a layer of kraut was removed for an analysis the first 6 inches was discarded before the sample for analysis was taken.

The total number of bacteria present at different stages of fermentation of the kraut were determined by the dilution method, the plate method, and the direct count or Breed method.[5] The organisms present were classified in groups according to their ability to ferment various sugars, mannitol, and litmus milk. Yeast water containing 1 per cent of these compounds was used as a culture medium.

The chemical work consisted of determining the amounts of volatile and nonvolatile acid and alcohol and the total amount of sugar in the brine. The methods used have been described in previous publications.[6]

DETERMINATION OF THE NUMBER OF BACTERIA BY THE PLATE METHOD

To determine the number of bacteria present, glucose-yeast-water agar plates were poured from dilutions of kraut brine made in the usual manner (Table I).

Observations made on the plates at the time of counting showed marked differences in appearance at the various intervals of plating. There was also a noticeable difference in the flora on the plates made from uninoculated kraut. This was most noticeable during the first days of the fermentation. The plates made from the brine 1 day old showed a large number of molds of various types and many different forms of bacteria. Some of these formed large, slimy, raised colonies; some, thin spreading colonies; and others, chromogenic colonies ranging in color from gray or brown to orange and red. The chromogens and molds were present on the plates from both the inoculated and uninoculated kraut but did not appear on plates from dilutions greater than 10,000.

TABLE I.—*Number of bacteria in young sauerkraut as obtained by the plate method*

Age	Number of bacteria in 1 c. c. of brine	
	Uninoculated	Inoculated
Days	*Thousands* [1]	*Thousands* [1]
1	200	170 to 700
3	5,000 to 5,500	3,900 to 4,000
5	6,000 to 6,400	8,000 to 8,200
7	39,000 to 40,000	50,000 to 55,000

[1] Thousands—i. e., 000 omitted.

By the third day the flora had changed and practically only two types of bacterial colonies were present—one a small lens-shaped colony which grew beneath the surface, and the other a surface colony which had a solid center and a hazy border. The uninoculated kraut showed two or three spreading colonies per plate at a dilution of 1,000, but the plates made from the inoculated kraut showed no molds or spreading colonies. The molds, spreading colonies, and chromogens in the natural fermentation persisted for only four or five days. By the fifth day only two types of colonies were present on both control and inoculated plates—the small colony beneath the surface and the large colony on the surface. The small colonies were more abundant than the large colonies, and this ratio was more marked in the inoculated than in the uninoculated kraut.

THE NUMBER OF BACTERIA AS DETERMINED BY DIRECT COUNT OR BREED PLATE METHOD

Each time the vats were opened a sample of brine was removed with a sterile pipette and used for making

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counts by the Breed plate method. The formation of clumps makes it difficult to determine the number of single cells. In many cases clumps of bacteria may have been counted as one cell (Table II).

TABLE II.—*Number of bacteria in sauerkraut as obtained by the direct-count method*

Age	Number of bacteria in 1 c. c. of brine	
	Uninoculated	Inoculated
Days	*Thousands* [1]	*Thousands* [1]
3	55,000	60,000
5	80,000	85,000
7	105,000	145,000
12	115,000	125,000
21	110,000	120,000
30		125,000
73	50,000	50,000

[1] Thousands—i. e., 000 omitted.

The examination of the Breed plates showed, first, a change in the ratio between the types of organisms present in sauerkraut at different times during the fermentation; second, a difference in the flora of the inoculated and uninoculated krauts. During the early part of the fermentation period the inoculated kraut showed about equal numbers of short rods and coccus forms, while the uninoculated kraut contained more rods than coccus types. During the course of the fermentation the number of coccus forms decreased in relation to the number of rod forms. This decrease was most rapid in the uninoculated kraut. The short rods were later replaced by a long rod which was the predominating organism at the end of the fermentation. There was very little difference in the appearance of the flora of the uninoculated and inoculated kraut at the end of the fermentation, because the coccus forms had practically disappeared in both cases.

DETERMINATION OF THE NUMBER AND KIND OF BACTERIA BY MEANS OF SUGARS

Each time when the vats were opened, a small quantity of brine was removed from the vats with a sterile pipette and placed in a sterile flask. The brine was carried through a series of eight dilutions extending from 1 c. c., diluted to 100, to 1 c. c. diluted to 1 billion. From each of these dilutions 1 c. c. was transferred to a tube containing 10 c. c. of sterile yeast water and 0.5 per cent of one of the following compounds: Glucose, lactose, xylose, and mannitol. Besides the sugar media, two sets of tubes containing litmus milk were also inoculated in the same manner. One set of tubes of litmus milk was heated after inoculation to 80° C. for 10 minutes, and then melted vaseline was poured into the tube. All of the tubes were incubated for 96 hours at 28° C. Observations were made for turbidity and gas production after 24, 48, and 96 hours after inoculation. The highest dilution in which there was turbidity or production of gas was taken as the number of organisms present which would ferment that particular substance. After 96 hours the tubes were kept at room temperature, approximately 20° C., for 10 days, and at the end of this time the total acidity was titrated with 0.1 N NaOH.

The litmus milk tubes with vaseline plugs were made to determine if any spore-forming, gas-producing anaerobes were present. Since no gas was produced and the litmus milk was not reduced or curdled, it is believed that none of the above group of organisms was present in the kraut.

Table III has been compiled from more than 300 observations and shows the number of organisms in 1. c. c. of brine that will ferment some of the common sugars, mannitol, and litmus milk. The rate of increase and decrease in the number of organisms is expressed in the same table.

It will be noted that the number of bacteria which ferment any of the substances used increases rapidly during the first 7 days of the fermentation and then decreases. The number of mannitol fermenters is much less than the number of sugar fermenters at the height of the fermentation, but toward the end of the fermentation the numbers are approximately equal. The enormous number of xylose fermenters present in the kraut at the height of the fermentation shows the importance of these bacteria in kraut formation. The influence of inoculation is indicated by the smaller number of xylose fermenters found in the inoculated kraut. This is to be expected because the lactic bacteria used to inoculate the kraut do not ferment xylose.

The figures for litmus milk show that the lactic acid organisms increase much more rapidly in the inoculated than in the uninoculated kraut, and that they are present in much greater

TABLE III.—*Number of bacteria in sauerkraut that ferment various compounds*

[Thousands of bacteria in 1 c. c. of brine [1]]

No.	Medium	Treatment	Age in days						
			1	3	5	7	12	30	73
1	Litmus milk	Uninoculated	1	10	100	100	1	1	0.1
2	do	Inoculated	10	10,000	10,000	100,000	100	100	.1
3	Glucose	Uninoculated	10	10,000	10,000	1,000,000	100,000	1,000	10
4	do	Inoculated	100	10,000	100,000	1,000,000	10,000	10,000	100
5	Lactose	Uninoculated	10	1,000	10,000	1,000,000	10,000	10,000	10
6	do	Inoculated	10	1,000	10,000	1,000,000	100,000	100,000	100
7	Xylose	Uninoculated	10	10,000	10,000	1,000,000	100,000	10,000	100
8	do	Inoculated	10	1,000	10,000	1,000,000	100,000	1,000	10
9	Mannitol	Uninoculated	10	1,000	10,000	10,000	10,000	10,000	100
10	do	Inoculated	10	1,000	10,000	100,000	10,000	10,000	100

[1] Thousands—i. e., 000 omitted.

TABLE IV.—*Reduction and curdling time of litmus milk inoculated with diluted sauerkraut brine*

Kind of kraut	Age of kraut	Highest dilution for reduction and curdling	Reduction time	Curdling time	Kind of kraut	Age of kraut	Highest dilution for reduction and curdling	Reduction time	Curdling time
	Days		*Hours*	*Hours*		*Days*		*Hours*	*Hours*
Uninoculated	1	3	24	48	Inoculated	7	8	48	96
Inoculated	1	4	48	48	Uninoculated	12	3	24	48
Uninoculated	3	4	24	48	Inoculated	12	5	48	96
Inoculated	3	7	48	96	Uninoculated	30	3	48	96
Uninoculated	5	5	48	96	Inoculated	30	5	48	96
Inoculated	5	7	48	96	Uninoculated	73	2	48	48
Uninoculated	7	5	48	96	Inoculated	73	2	48	96

TABLE V.—*Acid production from high and low dilutions of inoculated and uninoculated kraut*

[0.1 N acid in 10 c. c. of culture]

No.	Treatment	Dilution	Age in days							
			1	3	5	7	12	30	73	Average
	Litmus milk:		*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*
1	Uninoculated	Low	13.3	20.4	20.0	12.8	13.0	12.1	13.8	15.1
2	Inoculated	do	10.9	22.2	18.0	16.2	16.1	12.8	16.8	16.1
3	Uninoculated	High	12.0	15.1	15.5	11.1	13.0	12.1	13.8	13.2
4	Inoculated	do	13.5	10.9	14.4	10.9	12.3	16.6	16.8	13.6
	Xylose:									
5	Uninoculated	Low	5.2	9.0	8.7	9.0	8.5	3.8	7.4	7.4
6	Inoculated	do	5.9	9.8	8.3	8.3	8.5	5.5	9.2	7.9
7	Uninoculated	High	6.7	5.5	6.7	8.4	7.0	4.3	5.1	6.2
8	Inoculated	do	5.0	5.6	6.5	8.9	8.1	6.6	5.1	6.5
	Glucose:									
9	Uninoculated	Low	6.6	7.1	8.6	8.5	6.9	3.4	9.0	7.2
10	Inoculated	do	5.3	7.9	7.1	8.4	7.3	4.5	8.3	7.0
11	Uninoculated	High	4.3	5.2	5.3	5.7	6.1	5.5	8.1	5.7
12	Inoculated	do	4.4	5.0	6.0	6.5	7.5	5.2	7.8	6.1
	Lactose:									
13	Uninoculated	Low	5.5	8.2	7.4	6.8	6.8	5.5	3.0	6.2
14	Inoculated	do	6.7	9.1	6.5	6.1	5.7	5.5	3.7	6.2
15	Uninoculated	High	2.8	4.1	5.3	5.3	6.8	4.7	3.8	4.7
16	Inoculated	do	2.2	4.4	4.7	5.2	6.5	5.0	5.5	4.8

numbers throughout the greater part of the fermentation period. The litmus milk is used as a measure of the number of lactic bacteria which were added, because these organisms form a firm smooth curd, free from gas and without whey. They also reduce the litmus milk before curdling. The highest dilution in which there was reduction followed by curd formation was taken as the number of lactic-acid bacteria present.

It will be seen from the data in Table IV that reduction and curdling of the litmus milk takes place at much higher dilutions with the inoculated brine than with the uninoculated. It will also be noted that in all but two cases the reduction time precedes the curdling time. In these two cases reduction probably preceded curd formation, but observations were not made at short enough intervals to determine exactly when the changes occurred with respect to one another.

ACID PRODUCTION BY THE BACTERIA OF KRAUT

It has been mentioned previously that the solutions of sugars, mannitol, and litmus milk which had been inoculated with diluted kraut brine were titrated with 0.1 N NaOH at the end of 10 days. A great number of titrations were made, but all will not be given here. Table V shows the acidities formed from low and high dilutions of uninoculated and inoculated kraut. This table shows that the acidities developed from any of the substances used is greater when that substance is inoculated with brine from low dilutions than when it is inoculated from high dilution. Individual titrations may show an occasional exception to this rule, but when an average of all the titrations for each substance is taken it will be found to be true. It was also found that for intermediate dilutions the acidities developed were between those for the low and high dilutions in over 80 per cent of the cases.

These data may be interpreted as indicating that in sauerkraut there is a complex flora of organism and that those organisms which are present in largest numbers are not the highest acid producers. Bacteria present in small numbers are eliminated as the dilutions are increased while the more numerous but low acid-producing bacteria remain. Another explanation is the associative action of the bacteria. In the lower dilutions a greater variety of organisms is found than in the higher dilutions. The organisms present in the higher dilutions may be incapable of producing high acidities due to the absence of certain beneficial but non-acid-producing bacteria.

CHEMICAL DATA

The bacteriological data obtained tend to show that the bacterial flora has been influenced by inoculation with selected lactic organisms and that these organisms predominate during the early part of the fermentation. If this is correct, there should be a difference in the composition of the inoculated and uninoculated kraut.

Table VI shows the chief fermentation products found in sauerkraut at different periods during the fermentation. Inoculated kraut shows the presence of the characteristic products of the added lactic-acid culture, especially during the early part of the fermentation. The inoculated kraut is consistently higher in lactic acid and lower in alcohol and acetic acid than the uninoculated kraut. This differ-

TABLE VI.—*Chief products found in sauerkraut at various stages in the fermentation*

Treatment of kraut	Age	0.1 N acid in 100 c. c. of brine	Volatile acid as acetic	Non-volatile acid as lactic	Alcohol as ethyl	Total reducing sugar as glucose
	Days	*C. c.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Uninoculated	3	76	0.20	0.58	0.21	1.76
Inoculated	3	74	.17	.60	.17	1.95
Uninoculated	5	104	.30	.76	.24	1.97
Inoculated	5	101	.22	.80	.20	2.08
Uninoculated	7	152	.30	.84	.25	1.90
Inoculated	7	160	.23	.91	.22	1.93
Uninoculated	12	183	.37	1.19	.54	.64
Inoculated	12	174	.36	1.32	.31	.91
Uninoculated	21	193	.31	----------	.62	.34
Inoculated	21	195	.28	1.57	.37	.96
Uninoculated	30	200	.27	1.52	.75	.33
Inoculated	30	197	.34	----------	.53	.72
Uninoculated	73	188	.26	1.50	.60	.23
Inoculated	73	184	.33	1.69	.48	.30

ence is particularly marked in the case of alcohol.

The destruction of sugar is slower in the inoculated than in the uninoculated kraut, although the total acidity is about the same. It is not improbable that fermentation continues for a longer period in the inoculated kraut and thus aids materially in producing a kraut of good flavor as well as high acidity.

Many more determinations could be given to show the influence of inoculation on the fermentation products, but this would be only a repetition of Table VI. This table is representative of the results obtained from more than 50 analyses of inoculated and uninoculated krauts.

QUALITY OF THE KRAUT.—When the vats were opened for the final analysis, the quality of the inoculated and uninoculated kraut, at the age of 73 days, was compared by 10 people who were familiar with sauerkraut. The kraut which had been inoculated was far superior to the uninoculated kraut in regard to flavor and color, although there was not such a noticeable difference in the texture. This observation compares favorably with those made in 50 other cases where the effect of inoculation was studied.

However, the results obtained in this paper must not be construed as implying that the authors recommend the general use of inoculation in the preparation of sauerkraut. The results are suggestive but not final. Many experiments on a factory scale must be conducted to determine not only the possibility but also the practicability of such a procedure.

SUMMARY

Inoculation of sauerkraut with selected cultures of lactic-acid bacteria altered the normal flora of kraut and gave an improved product. It reduced the number of foreign organisms and the duration of their existence in the fermentation.

The presence of the added bacteria was most apparent during the period of most active fermentation and could be detected by direct microscopic examination and by inoculation of litmus milk. A reduction and curdling of the milk, typical of the added bacteria, was obtained.

The effect of inoculation is seen also in the fermentation products. The inoculated kraut contained more lactic acid and less acetic acid, and less ethyl alcohol than the uninoculated kraut.

A PHYSIOLOGICAL STUDY OF MUCOR RACEMOSUS AND DIPLODIA TUBERICOLA—TWO SWEET POTATO STORAGE-ROT FUNGI[1]

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INTRODUCTION

Most of the investigations conducted thus far by the writer and his colleagues on the storage-rot fungi of sweet potatoes have been conducted with the various species of the genus Rhizopus, a group of fungi generally conceded to be very destructive to many vegetables and some fruits (*7*).[2]

Inoculation experiments showed that 27 different hosts were susceptible to decay by some of the species, two of which, *Rhizopus microsporus* and *R. chinensis*, attacked the hosts somewhat weakly. Likewise, the same species, with the exception of *R. microsporus* and *R. chinensis*, were found to cause a soft rot of sweet potatoes (*6*). Numerous isolations from various hosts infected naturally in storage and on the markets gave almost exclusively either *R. nigricans* or *R. tritici*, the former greatly predominating. Subsequent investigations (*11*) demonstrated that these two species were primarily responsible for the decay of sweet potatoes in storage, and at different temperatures in infection chambers, even when brought into competition with other species of the same genus. *Rhizopus nigricans* predominated at temperatures between 6° and 20° C. and *R. tritici* at 30° and above, with some overlapping at temperatures from 20° to 30°.

These organisms produce a watery soft rot and cause the host to decay quickly. Under favorable conditions sweet potatoes of an average size may be decayed in from three to five days. The cells are not entered by the fungus, at least in the earlier stages. Investigations (*4*, *5*) have shown that an enzyme, pectinase, is secreted and has the ability to dissolve the middle lamellae so that coherence of the cells is lost. A portion of this cell wall dissolving enzyme was shown to be exuded into the culture media (*8*), the remainder being retained by the mycelium. When the fungus is grown two or three days on a suitable medium such as sweet potato decoction, raw sweet potato disks 1 mm. thick are completely disintegrated in from two to four hours when immersed in a substrate after freed from the mycelial growth.

The organisms of the Rhizopus group studied both from the standpoint of their parasitism and physiology produce decay at temperatures from about 16° to 30° C. or higher, and cause a rapid destruction of their hosts. They differ essentially in this respect from such fungi as *Mucor racemosus* and *Diplodia tubericola*, the two organisms which form the basis for the discussions in this paper.

Mucor racemosus, although not so common or destructive as *Rhizopus nigricans* or *R. tritici*, is nevertheless frequently found on vegetables, especially sweet potatoes (*3*), when held at temperatures a little above freezing. As a matter of fact, this fungus seems to be of no economic importance except at low temperatures. If, for example, sweet potatoes are held for several weeks at low temperature, from one to several infection centers of *M. racemosus* may develop. This fungus causes a slow progressive rot of sweet potatoes. The tissue is rendered gray and somewhat stringy, but not so wet as by the rot caused by *R. nigricans*. A microscopic examination of the decayed tissue shows that the cells are separated along the line of the middle lamellae in a manner similar to cells of tissue decayed with *R. nigricans* and *R. tritici*.

Diplodia tubericola differs parasitically and physiologically from both *Mucor racemosus* and the various species of Rhizopus. It appears to have a great variety of hosts and to grow

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[2] Reference is made by number (italic) to "Literature cited," p. 968.

saprophytically on almost any woody substance. This fungus is most prevalent in the southern part of the United States, where it is a common cause of a storage rot of sweet potatoes known as the Java black rot. It produces a slow, comparatively dry rot of sweet potatoes, rendering them finally black, hard, and mummified. Investigations have shown that it will cause decay where the humidity of the surrounding air is relatively low. For example, inoculated sweet potatoes will decay if laid on a shelf in a room heated with warm air.

Mucor racemosus and *Diplodia tubericola* seem to differ physiologically and pathologically from the Rhizopus group, with which much of the work thus far has been done. The former causes decay only at low temperatures, and the latter at rather high temperatures and in a relatively dry atmosphere. In view of the striking dissimilarity parasitically between these two organisms and the different species of Rhizopus, they were studied physiologically with the hope of obtaining some facts useful or applicable to the keeping of the sweet potato in storage.

METHODS OF EXPERIMENTATION

The detailed methods employed in conducting the experiments are discussed later, certain modifications and variations being imperative to meet certain phases of the problem, therefore only a cursory discussion of the methods used is given here, certain details being left for discussion when the experimental data are presented.

The culture of *Mucor racemosus* used in these experiments was obtained from sweet potatoes kept at a low temperature at Arlington Farm, near Rosslyn, Va. After this organism had fruited it was "pure-lined" by culturing from a single spore. This pure-lined culture was employed in all the experiments.

The culture of *Diplodia tubericola* was obtained from some decayed sweet potatoes sent to the writer from Alabama in 1922. This organism fruited abundantly in culture and was later proved to be parasitic. It was "pure-lined" in a similar manner to that of *Mucor racemosus*. Both of these organisms were kpet in an active state of vegetative growth by frequent transfers to suitable culture media.

For the enzymatic work several different culture media were employed at various times. The one used for most of the investigations was sweet potato decoction, which medium was prepared according to the following formula: To peeled potatoes add double their weight of water, steam for 1 hour, then squeeze out the liquid through gauze; steam a second time, filter by suction through absorbent cotton and autoclave 20 minutes at 15 pounds pressure. The resulting solution is practically free of cellulose structures, but it does contain some starch and some sugars. These organisms, especially *Mucor racemosus*, make a good growth on this medium.

In studying the secretion and macerating action of pectinase, both the solution on which the fungus grew and the mycelium itself were employed. The fungus was usually grown in 2-liter Erlenmeyer flasks on whatever media were selected for study. The mycelium was carefully removed from the flasks and washed in running water for about 10 minutes, after which the water was squeezed out, the mycelium being subsequently treated with acetone and ether according to a method previously employed by Dox (*2*).

The mycelium after treatment was spread out in order to allow the ether to escape, after which it was bottled and stored until required for use. The mycelium was ground in fine quartz sand before it was employed for macerating experiments. The solution on which the fungus had grown was filtered through absorbent cotton, which removed any mycelium that might be suspended in it, and many of the spores. Toluene was used as an antiseptic. This procedure was frequently unnecessary, inasmuch as maceration was usually complete in a few hours or before any contaminating organisms could alter the results. If the experiment was continued for several hours, toluene was added to the solution. The preparation was then thoroughly shaken and the flask tightly stoppered. Controls prepared by steaming for 10 minutes in an Arnold sterilizer were run with all experiments.

All reducing sugars were determined according to the method of Clark (*1*).

Raw sweet-potato disks were employed in determining the macerating action of the enzyme pectinase. These were cut 1 mm. thick and 15 mm. in diameter from as near the center of a sound sweet potato as possible. Further details of the methods employed may be found in an article by Harter and Weimer (*5*) published in 1921.

EXPERIMENTAL DATA

Enzymes Studied

No attempt has been made to study all or any considerable number of the enzymes which might be produced by

these two fungi. At the outset it was merely planned to study pectinase production, or the secretion and action of the enzyme responsible for the dissolution of the middle lamellae. However, since there was no information available as to any of the enzymes produced by these two fungi, an attempt was made to demonstrate the presence of a few of the more common ones.

PECTINASE

MUCOR RACEMOSUS.—A series of preliminary pectinase experiments with *Mucor racemosus* grown on a modification of Czapek's nutrient solution at room temperature failed to demonstrate the secretion of the enzyme cytase. However, an examination of sweet potatoes decayed by this fungus at temperatures a little above freezing showed that the middle lamellae were dissolved somewhat in advance of the growing hyphae. The fact that decay occurred only at such low temperatures suggested the possibility that either the macerating principle was secreted only at low temperatures, or that it was produced only on certain substrates, or that both hypotheses were true.

Another preliminary experiment in which sweet potato decoction was employed showed that a substance which would completely macerate raw sweet-potato disks in from 6 to 8 hours was secreted into the substrate. A number of different media were later employed as substrates, the results of which will be discussed elsewhere.

A series of experiments was planned subsequently in which after inoculation 2-liter Erlenmeyer flasks containing about 500 c. c. of sweet potato decoction were incubated at a temperature of 5° C. Immediately upon the removal of the cultures from the incubator the substrate and the mycelium were prepared for use in accordance with methods already discussed. As soon as the raw sweet-potato disks were added to the system incubation was carried out at a constant temperature of 35° C.

In the first experiment the organism was grown for 37 days, at the end of which time the flasks were removed from the incubator and the macerating power of the enzyme in the solution on which the fungus grew and that retained by the mycelium was determined. The results showed that maceration of the disks suspended in the substrate was evident in 3 hours, well advanced in 6 hours, and practically complete in 7 hours. The disintegration by the mycelium was very much slower, maceration being only started at the end of 41 hours.

In another series of experiments eight flasks were inoculated at one time and incubated at a constant temperature of 5° C. Three of these flasks were removed from the incubator at the end of 16 days, 3 after 24 days, and 2 at the end of 44 days. The results showed that there was no measurable difference in the time required to completely macerate raw sweet potato disks in the solutions removed from the incubators at the end of the three different periods of growth. Maceration started in about 3 hours and was practically complete at the end of 7 or 8 hours. But the enzyme in 0.25 gm. of mycelium suspended in 25 c. c. of water required 18 to 24 hours to disintegrate the middle lamellae completely. These experiments were repeated several times by growing the mycelium at the same temperature, with no measurable difference in the results.

It has already been pointed out that decay of sweet potatoes by *Mucor racemosus* has been observed to occur only at temperatures a little above freezing. In view of this fact, experiments on pectinase production were first carried out by growing the mycelium at a temperature of 5° C., in which the enzyme was shown to be produced. Some of the enzyme was exuded into the substrate and some was retained by the mycelium.

Inasmuch as decay by this fungus does not usually occur at higher temperatures, experiments were designed to determine whether or not pectinase was produced when the organism was grown at a temperature of 30° C. The methods of experimentation were the same as those already presented for 5°. Without discussing these experiments in detail it may be said that although pectinase was produced at 30°, it was somewhat less active than when the fungus was grown at the lower temperature, for 1 to 2 hours longer were required to macerate raw sweet potato disks completely when immersed in the solution. The action of the enzyme from mycelium suspended in water, however, was considerably slower; in some cases 48 hours or more were required to completely dissolve the middle lamellae so that coherence was lost. It was further found that a considerable amount of the macerating principle was produced during a three days' growth of the fungus, resembling in this respect some of the species of Rhizopus (*9*).

The results of these investigations show that *Mucor racemosus* when grown in sweet potato decoction at 5°

and 30° C. produces a pectinase which, though not as active as that from *Rhizopus tritici*, will macerate raw sweet potato disks.

DIPLODIA TUBERICOLA.—Numerous attempts were made to demonstrate the production of pectinase by *Diplodia tubericola*. This fungus was grown in 10 different substrates, among them sweet potato decoction, and in no case could the secretion of a macerating principle be demonstrated. In this respect the organism differs essentially from the other fungi studied. In view of the negative results obtained, the investigations with this fungus need not be detailed.

AMYLASE

The presence of amylase was demonstrated by the use of potato starch-paste solution to which a weighed quantity of dried mycelium ground in quartz sand was added. Hydrolysis was carried out for 19 hours at a temperature of 35° C. The reducing sugars were determined quantitatively by the method of Clark (*1*). All starch-paste solutions were tested before the addition of the ground mycelium for the presence of reducing sugars, and in no case were any found. A control in which ground mycelium was added to the starch-paste solution and the enzymes inactivated by immediately steaming, was carried in each experiment. The reducing sugars of the control preparations were determined, the amount of sugar found being probably due to autolysis of the mycelium and to such starch digestion as would probably take place before inactivation was accomplished by the steaming. The reducing sugars found were calculated in milligrams per 10 c. c. of solution.

MUCOR RACEMOSUS.—The production of amylase by *Mucor racemosus* was demonstrated by the use of one-fourth gram of the ground mycelium in 50 c. c. of a 0.5 per cent starch-paste solution. The mycelium used was grown on sweet potato decoction for 37 days at a temperature of 5° C. After incubation for 19 hours at 35° the controls averaged 3.14 mgm. and the solutions containing the active enzymes 19.87 mgm. of reducing sugars in each 10 c. c. of solution. This seems to demonstrate clearly that the mycelium contains an enzyme which has the power to hydrolyze starch.

DIPLODIA TUBERICOLA.—The experiments with *Diplodia tubericola* were conducted the same as with *Mucor racemosus*, except that a 2 per cent starch-paste solution was used with 0.5 gm. of powdered mycelium in 50 c. c. of solution. The mycelium was grown for 8 days on Czapek's modified solution at 30° C. The controls averaged 2.133 mgm. and the active enzyme preparation 52.96 mgm. of reducing sugars for each 10 c. c. of solution.

INVERTASE

The production of invertase by *Mucor racemosus* and *Diplodia tubericola* was determined by the use of mycelium from the same source as that employed in the demonstration of amylase. The amount of saccharose hydrolyzed was measured quantitatively by a determination of the reducing sugars.

MUCOR RACEMOSUS.—To 50 c. c. of a 0.5 per cent saccharose solution was added one-fourth gram of powdered mycelium. After incubation for 19 hours at 35° C. the controls averaged 1.42 mgm. and the active enzyme preparations 16.47 mgm. reducing sugars per 10 c. c. of solution.

DIPLODIA TUBERICOLA.—With this organism 0.5 gm. mycelium in 50 c. c. of a 2 per cent saccharose solution was used. At the end of the incubation period the controls averaged 6.09 mgm. and the active enzyme solution 175.88 mgm. reducing sugars per 10 c. c. of solution. The amount of reducing sugars in the control is somewhat larger than that usually found, which may possibly be accounted for by autolysis, or by some of the saccharose having been broken down in heating to inactivate the enzyme, or both of these reasons may be adduced. The sugar solution alone before the powdered mycelium was added or heated contained only 0.95 mgm. of reducing sugars.

RAFFINASE

The secretion of raffinase by *Mucor racemosus* and *Diplodia tubericola* was demonstrated by the hydrolysis of raffinose by the powdered mycelium. One-fourth of a gram of the powdered mycelium was suspended in 50 c. c. of 0.5 per cent raffinose solution and incubated for 21 hours at 35° C. A steamed control was carried at the same time. At the end of the incubation period further hydrolysis was stopped by steaming the solutions in an Arnold sterilizer. The reducing sugars were determined quantitatively.

MUCOR RACEMOSUS.—The controls gave an average of 3.55 mgm. and the active solutions 13.75 mgm. of reducing sugars per 10 c. c. of solution, thus demonstrating the presence of raffinase.

DIPLODIA TUBERICOLA.—The enzyme raffinase, secreted by this organism, was able to hydrolyze more than twice as much of the sugar as that of *Mucor racemosus* in the same length of time. Only 0.71 mgm. of reducing sugars was obtained from the control, while an average of 31.28 mgm. was obtained per 10 c. c. of solution in the preparation containing the active enzyme.

CYTASE

DIPLODIA TUBERICOLA.—It was previously pointed out that *Diplodia tubericola* caused a slow dry-rot of the sweet potato, the tissue finally becoming hard and mummified. The cells of the decayed tissue do not separate in the same way as when the rot is caused by *Mucor racemosus* or *Rhizopus nigricans*. It was also shown that the enzyme pectinase, which is abundantly produced by Mucor and Rhizopus, is not produced by *D. tubericola*, at least within the limits of these experiments. These results, together with the fact that hyphae have been observed within the cells, suggested the possibility that the enzyme cytase may be produced.

The first attempt to demonstrate the production of cytase was made with mycelium grown on sweet potato decoction. The mycelium was treated in the usual way and added to a suspension of cellulose in water. The cellulose was prepared from filter paper according to the method of Scales (*12*). One gram of powdered mycelium was added to 50 c. c. of the cellulose suspension and incubated at 35° C. At the end of 48 hours the system was tested for reducing sugars and none were found. Another method used successfully by Kellerman (*10*) to demonstrate the production of cytase by *Penicillium pinophilum* was tried. A beef agar with cellulose added was used as a medium. This preparation in test tubes was inoculated with *Diplodia tubericola* on the surface and incubated at a constant temperature of 30°. These cultures were grown for several weeks, but there was no clearing of the agar. On the other hand, *Sclerotium rolfsii*, used as a control, cleared the agar almost to the bottom of the tube in the same length of time. The experiment was repeated, using carrot and sweet potato agar with cellulose added. After inoculation the cultures were held for a number of weeks at 30°, but there was no evidence of the production of cytase. In this experiment water was placed in the incubator in order to prevent the media from drying out too rapidly.

MUCOR RACEMOSUS.—This fungus produced no cytase when tested according to the above method.

INFLUENCE OF SUBSTRATE ON PECTINASE PRODUCTION, DRY WEIGHT, AND HYDROGEN-ION CONCENTRATION

Essentially the same methods were employed for both *Mucor racemosus* and *Diplodia tubericola*, some variations being necessary to meet the different habits of the two fungi. *M. racemosus* was incubated at 25° C. and *D. tubericola*, being a higher temperature form was incubated at 30°. Since the latter organism develops much more slowly, it was grown for a longer time.

The following culture media were employed: Prune, sweet potato, potato, carrot, turnip, and bean decoctions, beef bouillon, and Czapek's, Pfeffer's, and Richard's solutions. Czapek's solution was modified by the substitution of ammonium nitrate for sodium nitrate. Enough of each of the stock solutions was prepared at one time to carry out the duplicated experiments. The experiments were set up as follows: Fifteen 100 c. c. flasks were used for each organism, 50 and 35 c. c. of the medium being used in each flask for *Mucor racemosus* and *Diplodia tubericola*, respectively. As soon as the culture media were pipetted into the flasks they were autoclaved for 20 minutes at 15 pounds pressure. They were then held for a few days in the laboratory at room temperature. As soon as it was evident that none of the flasks were contaminated, 10 of each medium were inoculated and incubated at temperatures given above, the remaining 5 being covered with oiled paper to prevent evaporation and held as controls, that is, for hydrogen-ion determinations.

MUCOR RACEMOSUS.—At the end of five days' growth three flasks of each medium were removed and the contents of each made into one compound sample. Two flasks were then prepared from this solution, one of which, after steaming 10 minutes to inactivate the enzyme, served as a control. Raw sweet potato disks were added to the solution and a record made from time to time of the progress of maceration. None of the disks in the steamed controls were macerated. The production of pectinase was not entirely what might be expected. It was abundantly secreted on sweet-potato and string-bean decoctions after 23 hours, on carrot decoctions after 30 hours; and well advanced on turnip decoction after 30

hours. None was secreted on prune or potato decoction or when the fungus was grown on beef bouillon or on Czapek's, Pfeffer's, or Richard's solution. These latter media have consistently failed to stimulate the production of the enzyme. This result is not so surprising, since the synthetic media are not supposed to contain any of the pectic compounds, which have been found to exercise a more or less regulatory influence on the secretion of pectinase.

Eight days after inoculation the remaining seven flasks were taken off. The mycelium from the flasks of the same substrate was brought together and used for dry-weight determinations. The total dry weight of the mycelium from these seven flasks proved to be as follows (average two experiments): Prune decoction, 0.2632 gm., sweet-potato decoction, 0.5668 gm.; carrot decoction, 0.3224 gm.; potato decoction, 0.3513 gm.; turnip decoction, 0.4845 gm.; string-bean decoction, 0.3684 gm.; beef bouillon, 0.2502 gm.; Czapek's solution, 0.4253 gm.; Pfeffer's solution, 0.3600 gm.; Richard's solution, 0.4476 gm. These data show that a fair growth of mycelium was produced in all the culture media used. However, there seems to be very little if any correlation between the amount of dry material and the production of pectinase. The smallest amount of dry material was produced on prune decoction and in beef bouillon, the media in which pectinase was not secreted. On the other hand, Richard's and Czapek's solutions, in which pectinase is not secreted, produced a considerable quantity of dry matter.

The hydrogen-ion determinations were made of the used and unused solutions at the end of the growth period on the same day the solutions were removed from the incubator. The control flasks, which were wrapped over the top with oiled paper to prevent evaporation, were kept in the incubator beside the inoculated ones. When required for hydrogen-ion determinations the contents of the different flasks of each medium were collected into one compound sample. The hydrogen-ion concentration of all the vegetable decoctions, except prune, also that of beef bouillon, is decreased by an 8-day growth of *Mucor racemosus*, but that of Czapek's, Pfeffer's, and Richard's solutions is increased or left practically unchanged (Table I).

DIPLODIA TUBERICOLA.—The same media were used in studying this organism as were used with *Mucor racemosus*. No pectinase was produced by *Diplodia tubericola* on any of the media used, and in view of that fact none of these data will be given.

TABLE I.—*Hydrogen-ion concentration of the controls and used solutions after 8 days' growth of Mucor racemosus. Average of two experiments*

Solution	Control (P_H)	Inoculated solution (P_H)
Prune decoction	3.795	3.645
Sweet-potato decoction	5.075	7.28
Carrot decoction	4.965	7.42
Potato decoction	5.525	7.99
Turnip decoction	4.865	7.455
String-bean decoction	4.625	7.58
Beef bouillon	7.18	8.445
Czapek's solution	5.08	3.485
Pfeffer's solution	3.71	3.76
Richard's solution	3.595	3.60

For the determination of the influence of the medium on dry-weight production and hydrogen-ion concentration the cultures were incubated for 10 days at 30° C. The same methods of procedure for the determination of dry weight of the mycelium and of the hydrogen-ion concentration were followed as with *Mucor racemosus*. The results of these total dry-weight tests (average of two experiments) are as follows: Prune decoction, 3.925 gm.; sweet-potato decoction, 0.7200 gm.; carrot decoction, 1.4498 gm.; potato decoction, 0.4232 gm.; turnip decoction, 1.9966 gm.; string-bean decoction, 0.8683 gm.; beef bouillon, 0.9886 gm.; Czapek's solution, 1.9882 gm.; Pfeffer's solution, 2.4475 gm.; Richard's solution, 1.0316 gm.

The amount of dry matter produced by *Diplodia tubericola* varied in the different media. The largest amount of dry material was produced in prune decoction, in which *Mucor racemosus* made a rather small growth. These data show that a medium which is well suited for the growth of one organism may not necessarily be suited for the growth of another.

To ascertain the hydrogen-ion concentration of the solutions, the contents of all the flasks of the same medium were collected into one compound sample and the P_H value determined as for *Mucor racemosus*. The results are given in Table II.

From Table II it is seen that *Diplodia tubericola* decreased the hydrogen-ion concentration of all the vegetable decoctions and of beef bouillon. The hydrogen-ion concentration of the three synthetic media (Pfeffer's, Richard's, and Czapek's) was increased.

TABLE II.—*Hydrogen-ion concentration of the controls and the used solutions after 10 days' growth of Diplodia tubericola. Average of two experiments*

Solution	Control (P_H)	Inoculated solution (P_H)
Prune decoction	3.86	3.98
Sweet-potato decoction	4.66	8.49
Carrot decoction	5.11	8.62
Potato decoction	6.04	8.72
Turnip decoction	4.98	8.47
String-bean decoction	4.75	8.62
Beef bouillon	7.15	8.59
Czapek's solution	3.77	2.52
Pfeffer's solution	3.62	2.42
Richard's solution	3.65	2.11

DISCUSSION

The foregoing data show that within the limits of these experiments *Diplodia tubericola* does not produce a macerating principle at any of the temperatures tried. On the other hand, *Mucor racemosus* secretes an enzyme which will completely disintegrate the tissue of raw sweet potato disks in from 6 to 8 hours. The same media were used in these experiments as were employed in studying the influence of the substrate on pectinase production by *Rhizopus tritici* (*8*). In the case of the latter organism dissolution of the middle lamellae was complete in a shorter period of time. However, a comparison of the results of the investigations of these two organisms with respect to the time elements is not strictly justifiable, in view of the known differences in the growth habits of the two fungi. It is interesting to note, however, that both fungi are alike influenced by the substrate on which they grow.

Pectinase was produced in all of the vegetable media tried except prune and potato decoction. None was produced by either *R. tritici* or *M. racemosus* when grown on synthetic media (Czapek's, Pfeffer's, and Richard's), or on beef bouillon where glucose was used as a source of carbon.

The only conclusion seemingly to be drawn from these results is that there is a regulatory influence of the substrate. Were it not for the results obtained from prune and potato decoctions it might be assumed on theoretical grounds that the regulatory substance may be some of the pectic compounds. In the article cited above it was shown that although no pectinase was produced by *R. tritici* when grown in Czapek's nutrient solution with glucose as a source of carbon, it was secreted if pectin was supplied as the only source of energy. On the other hand, if the pectin were combined with glucose as the available supply of carbon, the action on raw sweet potato disks was very feeble.

The volume of growth can hardly be urged as a possible explanation, inasmuch as it has been found that when the macerating principle is secreted, this occurs very early in the growth of the fungus, before any considerable amount of mycelium has been produced. A demonstrable amount of pectinase is secreted by *R. tritici* in 6 to 7 hours of growth and by *Mucor racemosus* in 3 days. Although the growth on prune decoction was less than on the other vegetable media employed, it was nevertheless fairly good, and on potato decoction it was not exceeded to any considerable extent except on sweet potato and turnip. The dry weight of mycelium produced on the synthetic media was equal on an average to that on the vegetable media, and no pectinase was produced.

A comparison of Tables I and II shows that both *Mucor racemosus* and *Diplodia tubericola* have somewhat similar action on the hydrogen-ion concentration of the substrate. Prune decoction was not materially changed, but the acidity of all the other vegetable decoctions and of beef bouillon was decreased, and in all cases to the alkaline side of neutrality. The hydrogen-ion concentration of Czapek's, Pfeffer's, and Richard's solutions was increased by *D. tubericola*. *M. racemosus* caused no appreciable change in the hydrogen-ion concentration of Pfeffer's and Richard's solutions, but increased the acidity of Czapek's solution. These results agree only in part with those obtained with *Botrytis cinerea* (*13*), which increased the hydrogen-ion concentration of some of these vegetable media and decreased that of others.

A study of these data and those of *Rhizopus tritici* show that different fungi act differently under what may be considered similar conditions. One organism may increase and another decrease the hydrogen-ion concentration of the same medium. From these results it is evident that no sweeping generalizations can be made for all fungi from the results obtained from a few. Each organism must be assumed *a priori* to be physiologically different from all others.

The investigations already carried out and discussed above show that pectinase was produced when *Mucor*

racemosus was grown on sweet potato, carrot, and turnip decoctions, but not when grown on potato decoction. Only sweet potato tissue was used for macerating experiments, and it was found that it was not macerated when suspended in a solution of potato decoction in which *Mucor racemosus* had grown. It was suspected that the enzyme might be specific in that the potato would be decayed if the organism was first grown on potato decoction.

Sound sweet potatoes, potatoes, carrots, and turnips were inoculated by the well method from a four-days' old growth of *Mucor racemosus* on sweet potato and potato decoction. After inoculation the hosts were kept at a constant temperature of 5° C. for 32 days, then removed from the incubator and examined. All of the sweet potatoes were partially decayed and isolations gave pure cultures of *M. racemosus*. None of the carrots, turnips, or potatoes were decayed, which shows that *M. racemosus* is not parasitic on them, at least within the limits of these experiments. Whether or not potato decoction was used made no difference.

It has already been shown that *Mucor racemosus* when grown on turnip and carrot decoction secretes into the solution a substance which will macerate raw sweet potato tissue, although the fungus is not parasitic on either carrot or turnip. In view of this fact an experiment was outlined for the purpose of determining if when grown on sweet potato decoction a substance is produced which will macerate raw turnip and carrot disks. These experiments were carried out in the usual way by the use of 2-liter Erlenmeyer flasks, which after inoculation were incubated at 10° C. After 14 days of growth the solutions were freed of mycelium by filtering through absorbent cotton. Disks of raw sweet potato, carrot, and turnip 1 mm. thick were suspended in the solutions and incubated at 35°. The results showed that maceration was started in every case in about 3½ hours and was nearly complete in 5 hours. It is evident from these investigations that *M. racemosus* will secrete into the substrate a substance which will dissolve the middle lamellae of hosts of which it is not a parasite.

SUMMARY

The secretion of pectinase by *Mucor racemosus* which causes a rot of sweet potatoes at a temperature of 5° C. and lower, and by *Diplodia tubericola*, the cause of a slow dry-rot at higher temperatures, was tried on 10 different culture media. *D. tubericola* did not secrete the enzyme. *M. racemosus* produced it on certain vegetable decoctions (sweet potato, carrot, turnip, string bean), but not on prune or potato decoction, or on synthetic media (Czapek's, Richard's, Pfeffer's) and beef bouillon.

Both *Mucor racemosus* and *Diplodia tubericola* secreted the enzymes, amylase, invertase, and raffinase, but not cytase.

The influence of these two fungi on the hydrogen-ion concentration of the 10 substrates used was investigated with the following results: *M. racemosus* decreased the hydrogen-ion concentration of sweet-potato, carrot, potato, turnip, and string-bean decoction, and of beef bouillon, and increased that of prune decoction and of Czapek's modified nutrient solution. There was no appreciable change of Pfeffer's and Richard's solutions. *D. tubericola* decreased the hydrogen-ion concentration of all of the vegetable decoctions (prune, slightly) and of beef bouillon. The hydrogen-ion concentration was increased when grown on Czapek's, Pfeffer's, and Richard's solutions.

Mucor racemosus was found to be parasitic on sweet potatoes, but not on turnips, carrots, or potatoes. The growth of this organism on potatoes did not produce an enzyme which would macerate potato tissue. Although not parasitic on turnips and carrots, an enzyme which would macerate sweet-potato disks was produced when grown on decoctions made from these vegetables. When the organism was grown on sweet-potato decoction an enzyme which would macerate turnip and carrot tissue was secreted.

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MICROORGANISMS IN DECOMPOSING OYSTERS [1]

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INTRODUCTION

In a previous paper on the spoilage of oysters (*6*)[3] the total aerobic counts of bacteria were given for shucked oysters in various stages of decomposition. The results of that work indicated that there was no definite correlation between the total number of bacteria present and the stage of decomposition of the oysters. It was shown, however, that there was a definite correlation between the hydrogen-ion concentration of the oyster liquor and the stage of spoilage. Realizing that the spoilage of oysters was probably due to the presence and development of bacteria of certain groups and that after identification of the groups responsible for the spoilage it might be possible to establish a correlation between a differential count of bacteria of certain types and the stage of decomposition, it was apparent that the next step in the investigation was to collect and study, for identification, the bacteria present in decomposing oysters. This paper gives the results of a study of several hundred cultures collected from the dextrose-agar shake cultures and from the dextrose-agar and wort-agar plates made in the course of the investigation already reported (*6*).

NOMENCLATURE

The nomenclature for bacteria, as presented in Bergey's Manual of Determinative Bacteriology (*11*) by a committee of the Society of American Bacteriologists, is used throughout this report. An attempt has been made to identify the organisms to species, but, owing to the meager and inadequate descriptions often found in the literature and to the reactions of the peculiar atypical forms frequently isolated from material of the kind studied here, it has not been possible in all cases to definitely identify the organisms by specific names. The names given to some of the less common forms encountered, therefore, are those of the types most nearly resembled.

The studies of Levine (*8*), of Winslow, Kligler, and Rothberg (*15*), and of many others on the lactose-fermenting group of bacteria, the work of Edson and Carpenter (*2*) and of Tanner (*12*) on the fluorescent bacteria, the investigations of Wenner and Rettger (*14*) and of Bengtson (*1*) on the proteus group, and of Ford and his coworkers (*7*) on the aerobic spore-bearing bacteria, make it possible to identify rather definitely members of these groups. The common water and soil forms belonging to the genera Achromobacter, Serratia, and Flavobacterium do not lend themselves so readily to identification, and it is in these genera particularly that no claim is made that the organisms so named are actually true to type.

EXPERIMENTAL WORK

In collecting the cultures from the dextrose-agar and wort-agar plates, many duplicates were inevitably obtained. In order to eliminate these duplicates, the organisms were studied as to their morphology, Gram staining reaction, growth on agar and gelatin, and reactions in litmus milk and in dextrose, lactose, maltose, and sucrose broths. After the elimination of the duplicates, for the purpose of more definite identification, the following characters of the cultures were also determined: Motility; indole production; reduction of nitrates; fermentation of additional carbohydrates, whenever necessary; reaction to the methyl-red test and the Voges-Proskauer test, whenever significant; growth on potato; and the production of hydrogen sulphide. An accurate record was kept of the history of each organism in order that some data might be available on the source and treatment of the oysters from which the organism was isolated, its relative abundance in the decomposing oysters, and the stage of spoilage at which it was isolated.

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[2] Thanks for valuable suggestions and criticisms are expressed to Charles Thom, Mycologist in Charge, Microbiological Laboratory.

[3] Reference is made by number (italic) to "Literature cited," p. 975.

Several experiments were conducted to investigate the power of the various pure cultures to produce typical spoilage in raw shucked oysters.

Clean oysters in the shell, obtained from unpolluted sources, were "floated" in the laboratory in tanks of artificial sea water to which had been added calcium hypochlorite in sufficient quantity to produce 6 parts of free chlorine per 1,000,000 parts of water. Following the directions given by Wells (*13*) for artificial purification of oysters, an attempt was made on a laboratory scale to reduce the bacterial content of the oysters to a minimum. After a suitable period in the chlorinated water, the oysters were opened aseptically, and one or two oysters, with their liquor, were placed in each of a sufficient number of large sterile test tubes. These chlorinated oysters were then inoculated with saline suspensions of the various pure cultures in the collection, grown for 48 hours on dextrose-agar slants. The inoculated oysters were held at 20° to 25° C., and daily records were made of their odor, appearance, and hydrogen-ion concentration. This experiment was repeated several times. Although not entirely successful, it furnished data which, when considered with some supplemental results from another experiment, presumptively identified those organisms in the collection which were responsible for the spoilage of shucked oysters.

In order to confirm the results obtained in these inoculation experiments, an oyster-infusion broth was prepared from chopped oysters, tubed, and sterilized in the autoclave. Before sterilization a small piece of oyster meat was added to each tube of plain oyster infusion. These tubes were inoculated with the various pure cultures, and the same series of observations made in the inoculation experiments were recorded.

EXPERIMENTAL RESULTS

From the combined results of these several experiments, it was apparent that certain of the bacteria used produced foul, putrefactive odors in the oysters, some produced acidity or sour odors, and others apparently produced neither putrefaction nor souring in pure culture, although they grew readily.

Table I gives the types of bacteria isolated from decomposing oysters which, when inoculated into artificially purified oysters or into oyster infusion medium, produced foul and putrefactive odors. Table II shows the types of organisms from oysters which produced acidity or sourness in oysters and in oyster infusion medium. Table III includes the remaining organisms isolated from decomposing oysters which apparently had no effect in pure culture.

The data in Table I show that the bacteria producing foul odors in decomposing oysters are members of the genera [4] Serratia (water and soil bacteria producing red pigment), Pseudomonas (soil and water bacteria producing a blue-green pigment), Proteus, Clostridium (spore-forming obligate anaerobes), and Bacillus (aerobic spore-forming bacteria). The first four groups were always present during the course of the spoilage and were always comparatively abundant and active. The spore-forming aerobic bacteria were always obtained during the early stages of the decomposition, and they did not appear to be as abundant or as active in producing evidences of spoilage as were the other organisms named in Table I.

The organism designated in Table I as CS–8c, which evidently belongs to the genus Serratia, has been isolated repeatedly, not only in this investigation but in an investigation on the decomposition of salmon (*5*). Since this organism appears to be a particularly active agent in the decomposition of fish and shellfish and since it can not be identified as any member of the genus Serratia described in the literature available, its morphology and cultural reactions may properly be given here. This organism is a Gram negative motile rod with bipolar flagella, 1.8 to 3 microns long and about 1 micron wide. Gelatin colonies are small, white, and perfectly round. Agar colonies are round, of medium size, and yellowish in the center, with bluish edges. Gelatin is rapidly liquefied, the growth being mostly on the surface of the stab. On agar slants the organism grows along the streak; it is pinkish, moist, and slightly raised. There is turbidity in plain broth, with a very slight scum and very little sediment in 48 hours. In old broth cultures there is a heavy viscous sediment, with a definite ring around the tube at the surface of the broth. Litmus milk is reduced and slowly peptonized, with the evolution of a foul odor. On potato the growth is heavy, moist, glistening, brick red, and slightly raised.

[4] Nomenclature follows Bergey's Manual (*11*).

TABLE I.—*Organisms producing foul odors in oysters and oyster infusion medium*

Culture (CS)	Number of times isolated[a]	Stage of spoilage when isolated	Species resembled
8c	29	Present throughout whole period of spoilage.	Serratia sp. (Bizio).
4G	17	do	Pseudomonas fluorescens (Flügge).
2C	27	do	Proteus vulgaris (Hauser).
1P	6	do	Bacillus cereus (Frankland).
3AN	20	do	Clostridium sp. (Prazmowski).
$5A_1$	24	do	Do.
$5A_4$	15	do	Do.
$6A_1$	8	do	Do.
1F	8	Present during early stages of spoilage	Bacillus subtilis (Ehrenberg) Cohn.
9G	2	do	Bacillus aterrimus (Lehmann and Neumann).
7	11	do	Bacillus simplex (Gottheil).
12A	2	do	Bacillus prausnitzii (Trevisan).

[a] In picking colonies from plates an effort was made to pick all the representative colonies from every plate examined.

There is no fermentation of dextrose, lactose, sucrose, or maltose. Indole is not produced. Nitrates are reduced to nitrites. Hydrogen sulphide is produced.

Four spore-forming obligate anaerobes were isolated in the course of the investigation. These organisms resembled one another closely, but were carried through the investigation as individual organisms. It has not been possible to identify these anaerobic bacteria as to species, but it has been determined that they are active agents in producing spoilage in oysters.

Although decomposing shucked oysters have a foul and putrefactive odor, their predominating odor and their appearance are such that they are usually referred to as "sour" oysters. In Table II are listed the organisms which produce this sour odor or produce acidity as indicated by the hydrogen-ion concentration.

All the organisms in Table II, in pure-culture inoculation of oysters and oyster infusion medium, produced hydrogen-ion concentrations varying from P_H 5.6 to P_H 4.4. *Aerobacter aerogenes* (*Bact. aerogenes*) and *A. cloacae* (*Bact. cloacae*) were present throughout the whole period of the spoilage. In pure culture these organisms produced not only a sour odor and an increase in acidity but also a foul and somewhat putrefactive odor. *Escherichia coli* (*Bact. coli*) and *E. communior* (*Bact. communior*) were isolated only during the early stages of spoilage. In pure-culture inoculation experiments these organisms produced results very similar to those produced by *A. aerogenes* and *A. cloacae*.

During the late stages of spoilage the organisms predominating in the spoiled oysters are streptococci, lactobacilli, and yeasts. In pure-culture inoculations the streptococci produce

TABLE II.—*Organisms producing acidity, sour odor, or both, in oysters and oyster infusion medium*

Culture (CS)	Number of times isolated	Stage of spoilage when isolated	Type resembled
7L	71	Present throughout whole period of spoilage	Aerobacter aerogenes (Escherich).
2N	21	do	Aerobacter cloacae (Jordan).
10E	21	Present during early stages of spoilage	Escherichia coli (Escherich).
14E	13	do	Escherichia communior (Durham).
20E	16	Present during late stages of spoilage	Lactobacillus sp. (Beijerinck).
$7A_{20}$	2	do	Streptococcus sp. (Rosenbach).
10Q	25	do	Streptococcus lactis (Lister).
$13A_3$	1	Present during early stages of spoilage	Bacillus mycoides (Flügge).
20c	5	Abundant during late stages of spoilage	Yeasts.
16P	5	do	Do.
16S	4	do	Do.
17A	4	do	Do.
1Z	4	do	Do.
NY_4	1	Isolated during late stages of spoilage	Do.
$20A_4$	2	do	Yeastlike fungus.

no pronounced odors, but they bring about a marked increase in acidity. The lactobacilli produce both a sour odor and an increase in acidity. These organisms, isolated from sour oysters, have been found to ferment dextrose, lactose, sucrose, maltose, arabinose, and trehalose. Some yeasts and yeastlike forms produce no odor in oysters and oyster infusion medium in pure culture. Some of the yeasts isolated do not ferment any of the carbohydrates and apparently play no part in the spoilage of oysters. The yeasts included in Table II, however, produce sour, yeasty, or aromatic odors, as well as an increase in acidity in the oysters. Owing to the difficulties in such work, no attempt has been made to identify the yeasts beyond an examination for ascospore formation. From the observations made it is apparent that most of the yeasts belong to the group of Torulae.

spoilage. Others, which were present at the beginning of the experiment, either disappeared or were overgrown by other groups. The yeasts were usually obtained during the late stages of spoilage. Although the organisms in this table apparently have no effect on oysters in pure culture, it is not improbable that under natural conditions they supplement the activities of the other organisms present in bringing about decomposition.

In studying oysters from polluted and unpolluted sources, Fuller (*4*) found that the bacterial flora of the oysters was almost identical with that of the sea water from which they were taken. His work and the earlier works of Wood (*16, p. 759–764*), Foote (*3*), and Sabatier, Ducamp, and Petit (*10*) are in close agreement with the findings of the investigation reported here. The identity of the flora of the oysters and of the sea water is often destroyed

TABLE III.—*Organisms producing only slight "off" odors or no objectionable odors in oysters or oyster infusion medium*

Culture (CS)	Number of times isolated	Stage of spoilage when isolated	Type resembled
2M	45	Present throughout whole period of spoilage.	Achromobacter raveneli (Chester).
1H	2	do	Achromobacter superficialis (Jordan).
3M	26	do	Achromobacter inunctum (Pohl).
6E	2	Isolated during early stages of spoilage	Achromobacter tiogense (Wright).
15L	1	Isolated during late stage of spoilage	Achromobacter stoloniferum (Pohl).
17c	7	Present throughout whole period of spoilage.	Achromobacter solitarium (Ravenel).
$1A_{13}$	1	Isolated during late stage of spoilage	Eberthella sp. (Castellani and Chambers).
2K	12	Present throughout whole period of spoilage.	Eberthella leporis (Sternberg)
3R	2	Isolated during late stage of spoilage	Flavobacterium aurantinum (Hammer).
3A	2	Isolated during early stage of spoilage	Flavobacterium sp.
7C	1	do	Flavobacterium annulatum (Wright).
9A	3	do	Flavobacterium sulfureum (Zettnow).
$14A_4$	1	do	Flavobacterium rigensis (Bazarewski).
17P	1	do	Flavobacterium deciduosum (Wright).
3B	14	Present throughout whole period of spoilage.	Micrococcus candicans (Flügge).
$5A_9$	1	Isolated during late stage of spoilage	Staphylococcus citreus (Passet).
$10A_{12}$	2	Isolated during early stage of spoilage	Micrococcus subflavescens.
$17A_1$	1	do	Micrococcus freudenreichii (Guillebeau).
1U	2	Isolated during late stages of spoilage	Pink yeast.
IX	1	do	Do.
19F	1	do	Do.
$19A_2$	1	Isolated during late stage of spoilage	Yeasts.
21D	1	do	Do.
NY_5	5	do	Do.
19E	1	do	Yeastlike fungus.
$13A_2$	1	Isolated during early stage of spoilage	Actinomyces.

The organisms in Table III, which were isolated from oysters and yet have no pronounced effect when inoculated into oysters or oyster infusion medium, are for the most part ordinary nonspore-forming water and soil bacteria. Some of these organisms persisted throughout the whole period of by handling under unsanitary conditions.[5] The organisms which have been found in oysters, some of which bring about spoilage, are common water and soil organisms. This accords with the conclusions reached regarding the source and characteristics of the bacteria causing decomposition in salmon.[5]

[5] Personal communication from P. B. Parsons, Bureau of Chemistry.

It is well known that oysters contain carbohydrate in the form of glycogen. Very few of the organisms isolated and studied in this investigation were able to attack pure glycogen. A great many of them, however, were able to ferment dextrose, maltose, or both. Mitchell (*9*) stated that, with approaching death, glycogenolysis takes place in the dying animal tissue, making dextrose and maltose available. In the investigation here reported, tests were conducted to demonstrate the presence of reducing sugar in freshly shucked oysters. The fermenting or souring of oysters, therefore, is due to fermentation of the available dextrose and maltose rather than to direct decomposition of glycogen.

The results of this study indicate that the decomposition of shucked oysters in the beginning is probably due primarily to the activities of some members of the Serratia, Pseudomonas, Proteus, Clostridium, Bacillus, Aerobacter, and Escherichia groups of bacteria. Later in the course of the spoilage, streptococci, lactobacilli, and yeasts find more suitable conditions for development, until in the very late stages of decomposition the high-dilution plates made from the oysters, which become very sour and putrid, contain almost exclusively colonies of these three groups of organisms. In such oysters the hydrogen-ion concentration is between P_H 5.0 and 4.6.

Washing the shucked oysters in fresh water or in brine as conducted in these experiments had no apparent effect on the character of the bacterial flora causing spoilage. The oysters employed in these experiments were divided into two lots. The shells of the oysters in one lot were scrubbed thoroughly with a stiff brush under running water. The shells of the other lot were left unwashed. The oysters in both lots were then shucked in their own liquor. The oysters of one batch from washed shells and of one batch from unwashed shells were drained, washed in a colander in running fresh water for a few minutes, allowed to stand in a pan of fresh water for 10 minutes, drained, and stored. The remaining batch from each lot of shell oysters was drained, washed in a colander in running fresh water for a few minutes, allowed to stand in a pan of 2.5 per cent salt solution for 10 minutes, drained, and stored. The organisms which were found responsible for the decomposition were isolated consistently from the washed as well as the unwashed oysters. In commercial practice the use of a "blower" might, in some slight degree, affect the character of the flora, although various investigators have reported the impossibility of removing bacteria from the digestive canal of the oyster by any method of washing. Regardless of the effect of washing on the bacterial flora, good commercial practice calls for a thorough washing of shucked oysters in a weak brine to remove dirt, pieces of shell, seaweed, and other foreign material, and to produce as clean a product as possible.

SUMMARY

The decomposition of shucked oysters is apparently due primarily to the presence and development of certain members of the following groups of bacteria: Serratia (water and soil bacteria producing red pigment), Pseudomonas (the fluorescent group), Proteus, Clostridium (the anaerobic spore-forming group), Bacillus (the aerobic spore-forming group), Escherichia (the colon bacteria), Aerobacter (the aerogenes group), Streptococcus, and Lactobacillus. Some decomposition is also caused by certain yeasts.

The streptococci, lactobacilli, and yeasts predominate in the late stages of spoilage, when the hydrogen-ion concentration is between P_H 5.0 and P_H 4.6, while the other forms predominate in the early stages of decomposition.

The remaining organisms, comprising the microflora of oysters, are, for the most part, ordinary nonspore-forming soil and water bacteria and yeasts.

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THE EGG-PRODUCING CAPACITY OF ASCARIS LUMBRICOIDES[1]

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INTRODUCTION

In connection with the life history of *Ascaris lumbricoides*, concerning which there have been important developments in recent years as a result of the researches of Stewart and other investigators, the question of the capacity for egg production is of considerable interest. It is well known that this worm is very fertile, but a great deal of uncertainty exists as to the number of eggs that a female may produce, the various estimates in the literature ranging up to as many as 80,000,000 eggs. The writer has undertaken to make an approximate count of the number of eggs, fully developed and in process of development, in the female Ascaris. Various investigators (Cloquet,[2] Eschricht,[3] Siebold,[4] Leuckart,[5] and others) have described the reproductive system of the female *Ascaris lumbricoides*, with more or less completeness, and only a brief review of certain aspects of its anatomy, important in relation to the question of capacity for egg production, need here be given.

SEX ORGANS OF FEMALE ASCARID

The two uteri branch off from the vagina at its inner end about 6 mm. from the vulva. Bakker [6] concludes from the study of numerous specimens that as the body length becomes greater the uterine length does not increase proportionately. She found that whereas ascarids 19 and 20 cm. long contained uteri 17 and 18 cm. in length, respectively, ascarids 32 and 35 cm. long had uteri 22 and 24 cm. in length, respectively. The uteri lie in a fairly straight course backward from the genital pore, the distal portion of each turning forward. At this distal end is situated the receptaculum seminis, beyond which is the oviduct and then the long filiform ovary which coils back and forth in the body cavity to such an extent that a cross section of the body shows 20 or 30 sections of the genital tubes. Each ovary measures 120 to 200 cm. in length, five to eight times the total length of the worm itself.

A histological study of these various sex organs of the female ascarid shows the steps in the development of the ova. The extremely delicate free or distal portion of the ovary (about 225 μ in diameter) is the germinal zone; it contains a mass of protoplasm with an abundance of nuclei, or germinal vesicles, scattered through it. At a distance somewhat farther from the free end of the ovary, the protoplasm gradually forms around the vesicle and distinct cells or ova begin to appear (fig. 1). Then follows the developmental zone of the ovary, in which the ova have elongated and arranged themselves in wreaths around a rachis, an axial protoplasmic cord which supplies them with nutriment. The ova are from 100 to 200 μ in length, and as viewed in a longitudinal section of the ovary are 9 to 10 μ broad at the outer end and somewhat narrower at the inner end, which is attached to the rachis (fig. 2). In cross section of the ovary they are about 7μ broad at the outer end and sharply pointed at the attachment to the rachis (fig. 3). The germinal vesicle is situated near the broad end of the ovum in its early development, when the ovum has a length of 100 μ (fig. 4), but later it gradually approaches the center, at

[1] Received for publication August 22, 1924; issued July, 1925.

[2] CLOQUET, J. ANATOMIE DES VERS INTESTINAUX. 130 p.; illus. Paris. 1824.

[3] ESCHRICHT, D. F. INQUIRIES EXPERIMENTAL AND PHILOSOPHICAL, CONCERNING THE ORIGIN OF INTESTINAL WORMS. Edinb. New Phil. Jour. 31: 314–356, illus. 1841.

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[5] LEUCKART, R. DIE MENSCHLICHEN PARASITEN. Bd. 2., illus. Leipzig und Heidelberg. 1876.

[6] BAKKER, C. R. OVER DE IDENTITEIT VAN ASCARIS LUMBRICOIDES EN ASCARIS SUILLA. Tijdschr. Vergelijk. Geneesk. 6: 160–230, illus. 1921.

which time the ovum is about 200 μ long (fig. 2).

The rachis disappears as the character of the ovary changes to form the oviduct, and the ova separate from each other and gradually assume a more or less oval form. They are fertilized by the sperm in the receptaculum seminis, and the shell is developed while they are in the posterior end of the uterus.

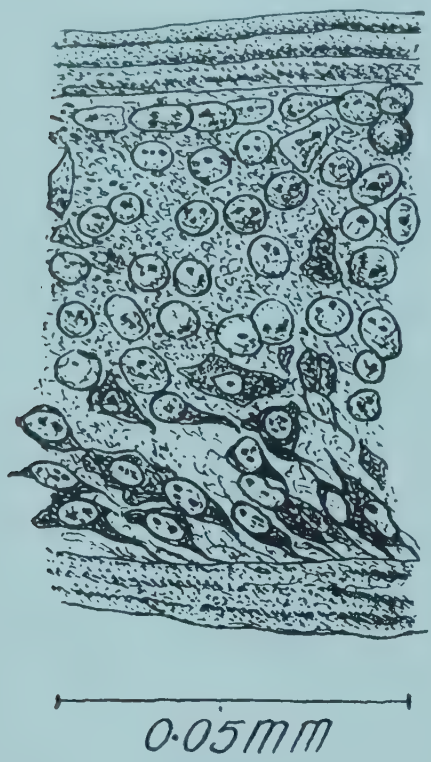

FIG. 1.—*Ascaris lumbricoides*. Germinal zone of ovary

METHOD OF COUNTING EGGS

The writer has attempted to count the eggs in two adult specimens of *Ascaris lumbricoides*. The genital system of the worm was carefully dissected, then floated in water while the many loops of tubules were disentangled, and then the uteri and ovaries were carefully measured. After that, portions were cut off at different levels, mounted in celloidin, and cross and longitudinal sections made of each portion of tubule. After staining and mounting the section, the eggs were counted in several sections from each of the selected portions, and the average computed. In the cross sections of ovary and uterus (figs. 3 and 5) the total number of eggs present was counted (see columns *a* and *a'* in the tables); in the longitudinal sections of ovary and uterus (figs. 2 and 6) a linear count was made of the eggs arranged consecutively along a known length of the wall of the tube, and from that length was computed the approximate number in a 1 mm. length (see columns *b* and *b'* of the tables). By multiplying the number of eggs in cross section by the linear count for

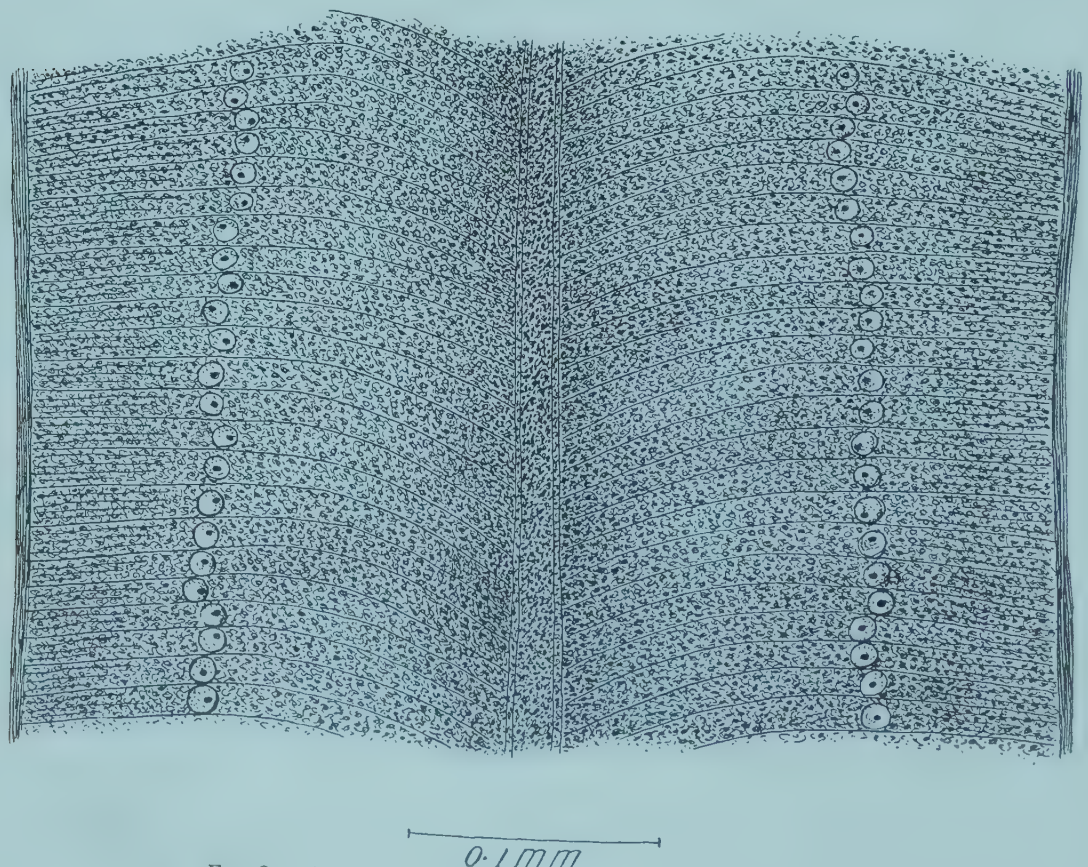

FIG. 2.—*Ascaris lumbricoides*. Longitudinal section of ovary

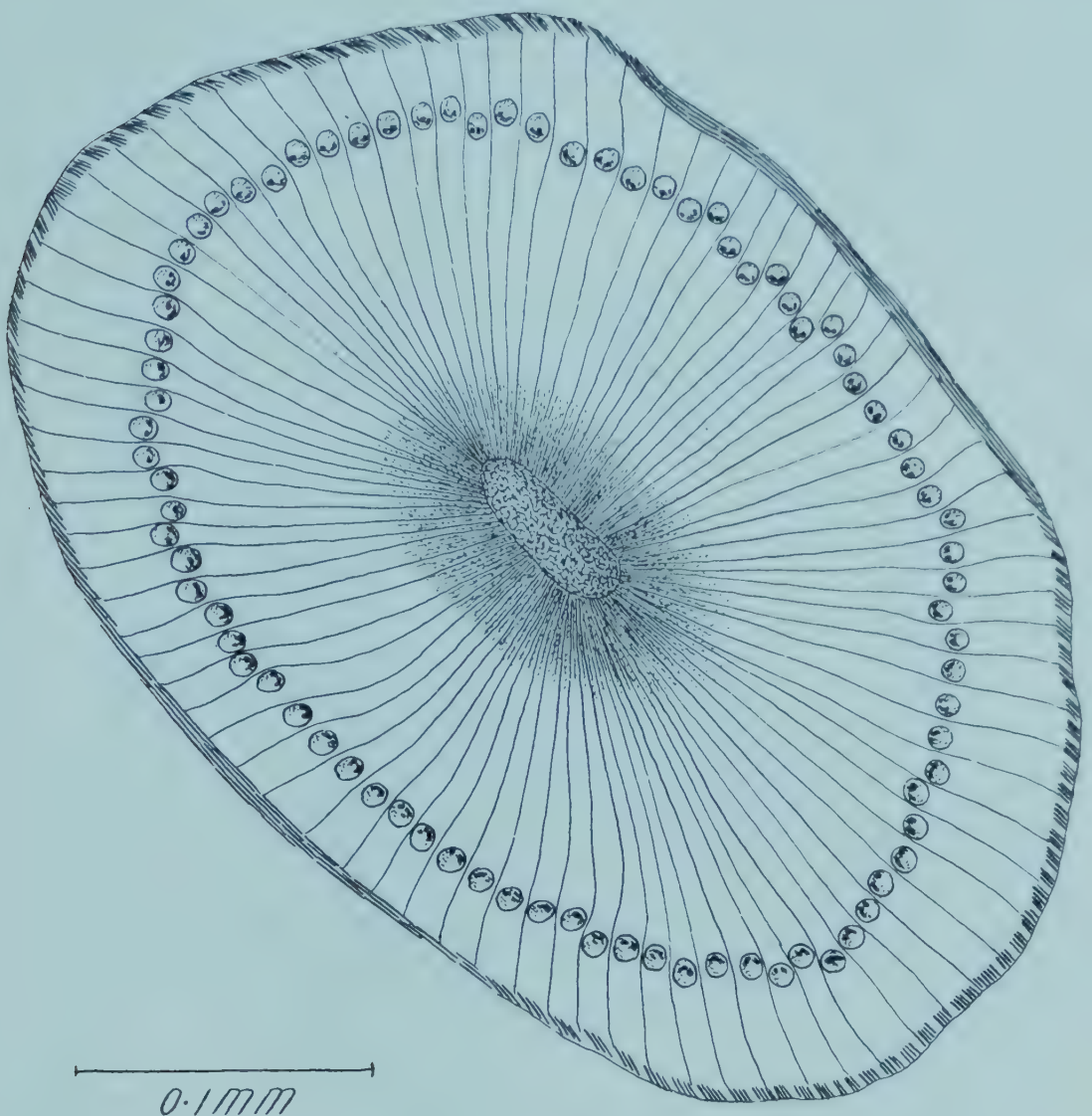

FIG. 3.—*Ascaris lumbricoides.* Cross section of ovary

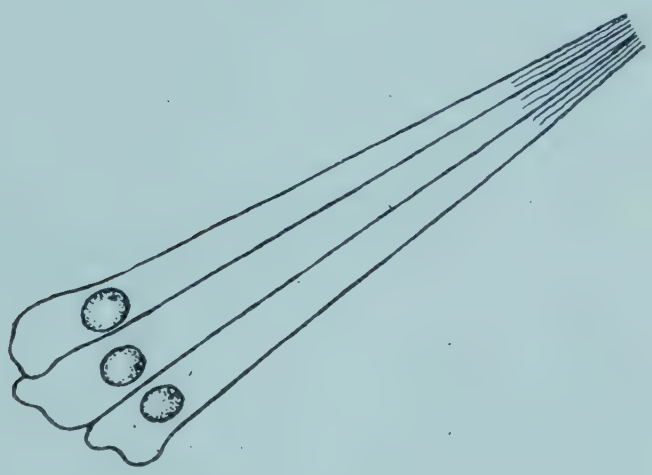

FIG. 4.—*Ascaris lumbricoides.* Ova from ovary

1 mm. longitudinal section, there was obtained the approximate total number of eggs in a 1 mm. length of the tube, either ovary or uterus (columns *c* and *c'* of the tables). Such counts were made of the first specimen (Table I, Ascaris A) on 5 portions of ovary (13 cross sections and 13 longitudinal sections being counted), and on 4 portions of uterus (12 cross sections and 12 longitudinal sections being counted). The average number of eggs in a 1 mm. length of ovary, as computed from the five parts selected, was 7,431.2; that in a 1 mm. length of uterus, as computed from the 4 parts selected, was 5,642.85. These numbers, multiplied by the total length of the ovaries and uteri, respectively, gave 23,668,372 as the total number of eggs in the ovaries, and 2,821,425 in the uteri, or a sum total of 26,489,797 eggs in that specimen of *Ascaris lumbricoides*.

The same procedure was followed with the second specimen, except that 18 cross and longitudinal sections of 6 portions of ovary and 9 cross and longitudinal sections of 3 portions of the uterus were counted (Table II, Ascaris B). The total number of eggs in the ovaries was 25,144,591 and that in the uteri 2,413,224, the total number in the worm being 27,557,815. For this second specimen a smaller worm than the first was deliberately chosen. The fact that the number of eggs in the

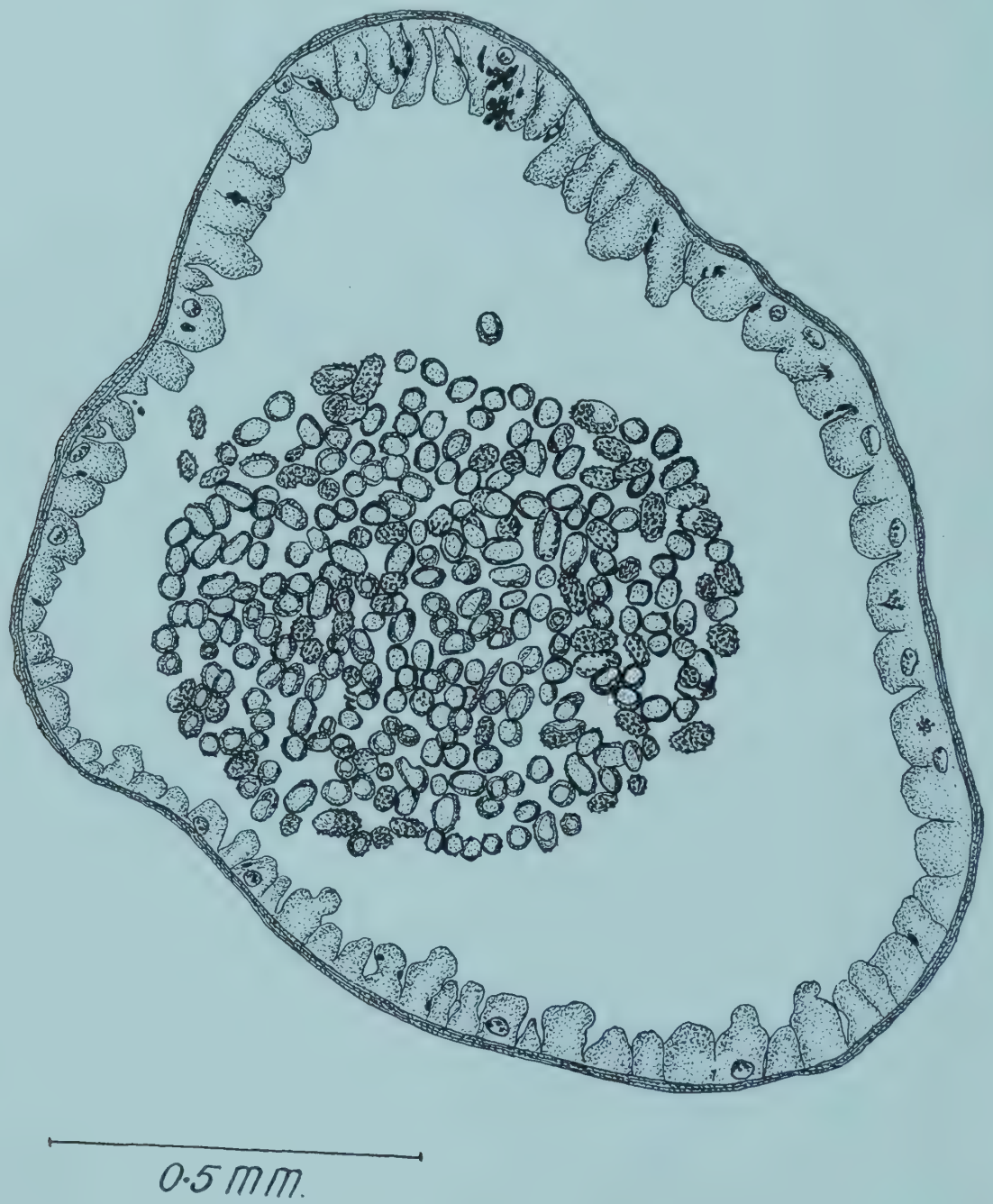

FIG. 5.—*Ascaris lumbricoides*. Cross section of uterus

ovaries, and especially in the germinal zone, of the smaller and therefore presumably younger female (Ascaris B) was greater than in the larger specimen (Ascaris A), while the number in the uteri of the younger worm was smaller and the total number of eggs in ovaries and uteri was slightly greater, is perhaps significant as indicating that in the older worm more eggs had matured and been deposited.

The free end of the ovary is but 1-25th of a line in diameter. A transversal section of the ovary shows the number of ova around the rachis to be about 50 and their diameter to be about 1-500th part of a line. Hence in the space of one line there will be 500 wreaths or stars of 50 eggs each, so affording 25,000 ova. The length of each horn of the female organ is about 16 ft. or 2,304 lines, which for the two horns gives 4,608 lines. If the ova, therefore, were of the same diameter throughout, their number would amount to 25,000×4,608, but as they augment in size as they proceed from the ovary to the uterus, till at last they attain a diameter of 1-60th of a line, they will not form more than 60 wreaths or 3,000 eggs in one line within the uterus. Thus, supposing the diameter of the eggs to increase proportionately throughout the length of the female organs, we may calculate the number of ova, on an average, at $\frac{25,000+3,000}{2}$, or 14,000 in each line, giving a total number of eggs at 14,000×4,608, of course more than 64,000,000.

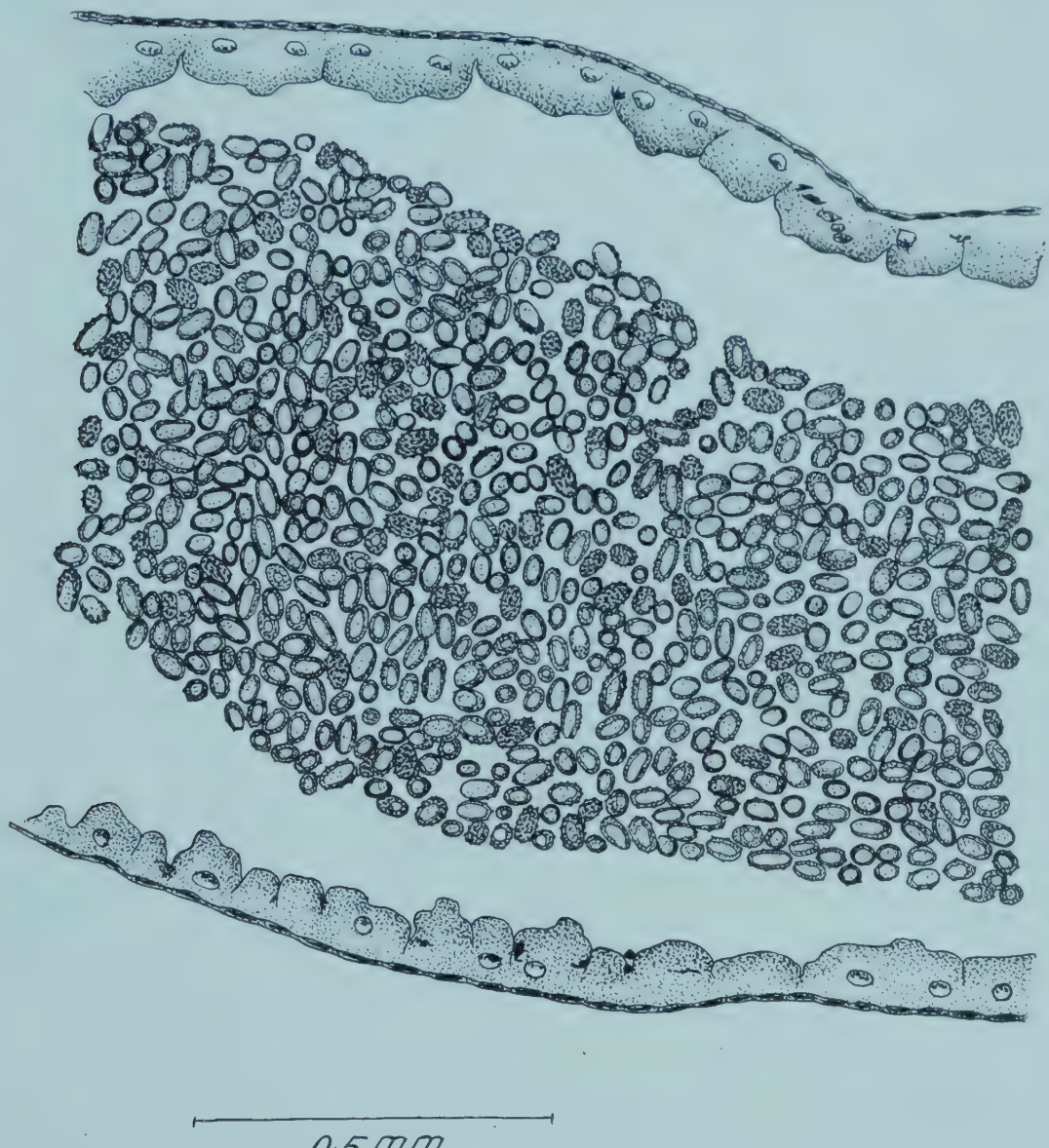

FIG. 6.—*Ascaris lumbricoides.* Longitudinal section of uterus

DISCUSSION OF RESULTS OF OTHER INVESTIGATORS

The figures obtained in these counts, 26,000,000 and 27,000,000 as the total number of eggs in a female ascarid, are considerably lower than those obtained by Eschricht (footnote 3) and by Leuckart (footnote 5), namely 64,000,000 and 70,000,000, respectively.

Eschricht's computations are as follows:

Eschricht evidently assumes in these computations that there are still 50 eggs in a cross section of the uterus as there were in the ovary, arranged around the rachis; this is indicated in his statement that the ova "will not

TABLE I.—*Ascaris* A. *Egg count in female 29 cm. long*

Ovaries (3,185 mm. combined length)			Uteri (500 mm. combined length)		
a In cross section	*b* Consecutive in 1 mm. longitudinal section	*c* Total in 1 mm. length of ovary ($a \times b$)	*a* In cross section	*b'* Consecutive in 1 mm. longitudinal section	*c'* Total in 1 mm. length of uterus ($a' \times b'$)
	Eggs	*Eggs*		*Eggs*	*Eggs*
I. Germinal zone:			I. 122	21.2	
35	144.9		114	17.7	
48	125.3		144	23.7	
Average 42	Average 135.1	5,674.2	Average 126.7	Average 20.9	2,648.0
II. 88	66.2		II. 571	27.3	
82	72.2		536	24.4	
91	72.2		545	23.6	
Average 87	Average 70.2	6,107.4	Average 550.7	Average 25.1	13,822.6
III. 75	65.8		III. 237	21.6	
78	82.3		221	21.3	
75	88.0		225	22.9	
Average 76	Average 78.7	5,981.2	Average 227.6	Average 21.9	4,984.4
IV. 76	111		IV. 45	24.7	
78	125		42	25.5	
Average 77	Average 118	9,086.0	46	25.3	
			Average 44.3	Average 25.2	1,116.4
V. 97	110.4		Average		5,642.85
93	102.6				
95	112.4				
Average 95	Average 108.5	10,307.5			
Average		7,431.2			

Total number of eggs in ovaries (7,431.2×3,185), 23,668,372.
Total number of eggs in uteri (5,642.85×500), 2,821,425.
Total number of eggs in the ascarid, 26,489,797.

TABLE II.—*Ascaris* B. *Egg count in female 27.5 cm. long*

Ovaries (3,115 mm. combined length)			Uteri (360 mm. combined length)		
a In cross section	*b* Consecutive in 1 mm. longitudinal section	*c* Total in 1 mm. length of ovary ($a \times b$)	*a'* In cross section	*b'* Consecutive in 1 mm. longitudinal section	*c'* Total in 1 mm. length of uterus ($a' \times b'$)
	Eggs	*Eggs*		*Eggs*	*Eggs*
I. Germinal zone:			I. 320	18.2	
148	123.4		376	19.8	
128	145.7		326	21.9	
185	127.6		Average 340.6	Average 19.9	6,777.9
Average 153.6	Average 132.2	20,305.9	II. 474	16.8	
II. 41	87.9		442	21.7	
41	86.0		462	22.1	
41	90.0		Average 459.3	Average 20.2	9,277.9
Average 41	Average 87.9	3,603.9	III. 306	16.8	
III. 56	69.2		218	15.9	
56	65.3		287	12.3	
60	72.2		Average 270.3	Average 15.0	4,054.5
Average 57.3	Average 68.9	3,948.0	Average		6,703.4
IV. 62	120.4				
62	108.7				
64	115.1				
Average 62.6	Average 114.7	7,180.2			
V. 67	115.6				
65	91.0				
68	110.1				
Average 66.6	Average 105.6	7,032.9			
VI. 54	110.8				
60	115.6				
56	110.8				
Average 56.6	Average 112.4	6,361.8			
Average		8,072.1			

Total number of eggs in ovaries (8,072.1 × 3115), 25,144,591.5.
Total number of eggs in uteri (6,703.4 × 360), 2,413,224.0.
Total number of eggs in the ascarid, 27,557,815.5.

form more than 60 *wreathes* in one line within the uterus." The eggs in the uterus are no longer the slender, elongate ova arranged radially around a rachis but are mature and unattached, and the counts in a cross section are seen in the present investigation to vary greatly, from 42 to 571 in such a section. Therefore, whereas Eschricht gives 3,000 eggs to a line, or, assuming that Eschricht's line was one-twelfth of a Paris inch of 27 mm. as used in Germany at the time his paper was published, 1,333 eggs to a 1 mm. length, in the uterus, the present writer found 5,642 and 6,703 to a 1 mm. length in the 2 specimens used.

The greatest difference, however, in the data given by Eschricht and those of this investigation is in the total lengths of the genital systems. Eschricht gives the length as 4,608 lines, which would be approximately 10,408 mm. The combined lengths of ovaries and uteri in the 2 specimens considered in this paper, however, were 3,684 mm. and 3,475 mm., respectively. In 3 other specimens measured by the writer the measurements were as follows: A female ascarid 28 cm. long had total uterine length of 360 mm. and total ovarian length of 3,520 mm.; in a specimen 28.5 cm. long the figures were 440 mm. and 3,650 mm., respectively; in a specimen 29 cm. long the figures were 460 mm. and 3,840 mm., respectively. Leuckart gives the length for a smaller worm (15.7 cm. in length) as 300 to 500 mm. for the combined length of uteri, and 2,500 for that of the ovaries. It seems then that Eschricht's measurements, two and one-half to three and one-half times as great as the above figures, are probably incorrect.

Leuckart computes the number of ova in an ascarid in the following manner. He states that the length of the ovary was 1,200 (presumably 1,200 mm.), the base of each cone-shaped ovum in it 0.04 mm., and the number of ova in a cross section 100. From these data he concludes that the total number of eggs in each ovary would be 30,000,000, or 60,000,000 for both. The uteri, he says, contain an additional 10,000,000 to 11,000,000 eggs. There is apparently some error in Leuckart's computations, as the above data would give 3,000,000 and 6,000,000 instead of 30,000,000 and 60,000,000. It is possible, however, that the discrepancy lies in the figure 0.04 mm. as the base of the ovum; none of the ovarian ova measured by the present writer exceeded 10 μ, the range being 6.5 to 10 μ, so that it is possible that Leuckart meant 4 μ instead of 40 μ, in which case his 30,000,000 and 60,000,000 result would be mathematically correct.

Another important difference between his counts and the present writer's is that for the cross section of the ovary Leuckart states that there were 100 or more ova around the rachis (although his figure shows only 48), whereas the counts in the present investigations varied considerably in different parts of the ovaries and in the two different specimens. In Ascaris A there was an average of 84 ova, and in Ascaris B an average of 57 in such sections. Leuckart does not seem to have taken into consideration the germinal zone of the ovary in which no rachis is present.

It is seen thus that both the computations of Eschricht and those of Leuckart are confused, apparently containing either incorrect observations or deductions or, as far as it is possible to judge from the data, being mathematically unsound, and their counts of 64,000,000 and 70,000,000, respectively, can not be accepted as completely trustworthy. It was because of this that the present investigation was undertaken and the counts made that are recorded herein.

SUMMARY

This study of American material of *Ascaris lumbricoides* indicates that the total number of eggs, fully developed and in process of development, in the female is from 26,000,000 to 27,000,000, and the former estimates of Eschricht and of Leuckart, 64,000,000 and 70,000,000, respectively, are apparently incorrect.

A STUDY OF ESSENTIAL PLANT FOODS RECOVERABLE FROM THE MANURE OF DAIRY COWS [1]

By C. F. Wells and B. A. Dunbar, *Chemistry Department, South Dakota State College*

INTRODUCTION

The problem briefly discussed in this article grew out of certain queries suggested by analytical results which the writers obtained during the progress of their work done in cooperation with the Dairy Department of South Dakota State College upon the determination of digestion coefficients which characterized dairy cattle of varied types under the feeding of soybean hay and alfalfa hay as variants.[2]

The work here reported offers a measure of direct information on the important subject of the degree of recovery of potash, phosphorus, and nitrogen from the manures of dairy cattle during lactation periods. These results also afford a general comparison between the quantities of potassium, phosphorus, and nitrogen recoverable in the manure and the amount of milk simultaneously produced by the same cattle. This work is by no means complete, but it is felt it is capable of expansion into broader research leading to more complete generalization than was possible with the limited number of subjects used in these digestion trials.

EXPERIMENTAL DATA

These digestion trials were conducted during two periods of five days each; the first in February and the second in April of 1924. For the trials four dairy cows, in lactation, were fed corn silage, oats, corn, and oil meal, as constants, in approximately the same quantities of each per day. The variant feeding material consisted of constant daily rations of soybean hay during the first trial and of alfalfa hay during the second. Each of these feeding materials, both constants and variables, was from the same lot originally purchased for the experiments, and therefore only one analytical problem was presented in case of each of them.

The cattle used as subjects were of the following breeds, and are hereinafter referred to by numbers as assigned here: Cow No. 1, Jersey; cow No. 2, Ayrshire; cow No. 3, Guernsey; cow No. 4, Holstein. All were of the ordinary farm grade of cows, of about the same age, ranging closely around 5 years. At the beginning of the trials all were apparently in perfect health, and they were considered to be in equally good condition at the close of the experiment. At all times during the trials, as well as during the several weeks of preliminary conditioning to accustom them to the constant feeds and the methods of manure collection, the cows were allowed free access to salt and water and were cleanly bedded and stalled in comfort. This careful attention was also given in the interim between the trials.

During the trials the feces and urine were carefully and thoroughly collected during 24-hour periods, and a composite sample of each collection was kept in air-tight glass containers. The five composites for each 5-day trial period were analyzed at the close of the period. The refuse feeding material was also collected at the close of the trial period and analyzed for the three plant-food constants, to be used as a drawback upon the amount of the several materials fed in obtaining actual consumption of feed per day. In computing the weights of each constant actually consumed per day, the totals for the trial periods were divided by five, and they are given on this basis in the tables. In the analyses of feeds and liquid and solid manures the writers, with but little deviation, followed recognized methods of analysis, and for their immediate purposes only nitrogen, potassium oxide, and phosphorus (as the pentoxide) were considered. One-hundred-gram samples were put into solution by the use of concentrated sulphuric acid of specific

[1] Received for publication Aug. 18, 1924; issued July, 1925.

[2] This work was in part fulfillment of requirements for the degree of Master of Science, and was expected to show the relative feeding values of the hays in question. The writers are therefore indebted to the dairy department for certain data from which, by further analytical investigation, the material for this paper has been obtained.

gravity 1.84 and under gentle heat. The original moist composites were used to obviate loss of nitrogen on drying and for comparison of the data with those on the urine composites. This preliminary solution was made up to volume with distilled water, and aliquots of the diluted solution were used for analysis. Potassium oxide was determined by the perchlorate method, phosphorus pentoxide by the volumetric molybdate method, and nitrogen by the Kjeldahl process. All results so obtained are reported in the following tables:

TABLE I.—*Analysis of feeds, original moist basis*

	K_2O	P_2O_5	N
	Per cent	*Per cent*	*Per cent*
Soybean hay	2.290	0.490	1.344
Alfalfa hay	2.260	.960	2.660
Corn silage	.300	.146	.388
Oats	.540	1.140	2.016
Corn	.407	.850	1.596
Oil meal	1.270	1.860	5.656

At the close of the first 5-day digestion trial, the refuse was carefully collected and weighed and a composite sample analyzed. This refuse was found to be almost wholly the stems of soybean hay. At the close of the second trial period there was practically no refuse obtainable from the stalls, so only one analysis is reported. Results are given in terms of average pounds per day.

TABLE II.—*Analysis of refuse feed, first digestion trial*

Cow No.	Refuse feed	K_2O		P_2O_5		N	
	Lbs.	*Lb.*	*P. ct.*	*Lb.*	*P. ct.*	*Lb.*	*P. ct.*
1	0.345	0.0030	0.90	0.0004	0.12	0.0022	0.64
2	.528	.0048	.90	.0006	.12	.0030	.58
3	.806	.0072	.90	.0010	.12	.0088	1.10
4	1.600	.0144	.90	.0018	.12	.0018	.12

Using the data in Tables I and II, the quantities of the several materials fed per day being known by reference to the feeding tables for the two trials, the data given in Tables III and IV are easily computed. These tables present the actual amounts of each of the three constants fed, wasted, and consumed during the two trials.

TABLE III.—*Computation of oxides of potassium, phosphorus and nitrogen in feed*

FIRST DIGESTION TRIAL

Feed	Cow No. 1, Jersey				Cow No. 2, Ayrshire				Cow No. 3, Guernsey				Cow No. 4, Holstein			
	Feed per day	K_2O per day	P_2O_5 per day	N per day	Feed per day	K_2O per day	P_2O_5 per day	N per day	Feed per day	K_2O per day	P_2O_5 per day	N per day	Feed per day	K_2O per day	P_2O_5 per day	N per day
	Lbs.	*Lb.*	*Lb.*	*Lb.*	*Lbs.*	*Lb.*	*Lb.*	*Lb.*	*Lbs.*	*Lb.*	*Lb.*	*Lb.*	*Lbs.*	*Lb.*	*Lb.*	*Lb.*
S. B. hay	14	0.3206	0.0686	0.1882	10	0.2290	0.0490	0.1344	10	0.2290	0.0490	0.1344	12.8	0.2930	0.0628	0.1720
Silage	30	.0900	.0438	.1164	25	.0750	.0364	.0970	25	.0750	.0364	.0970	30	.0900	.0438	.1164
Oats	4	.0218	.0456	.0806	4	.0218	.0456	.0806	4	.0218	.0456	.0806	4	.0218	.0456	.0806
Corn	5	.0204	.0424	.0798	5	.0204	.0424	.0798	4	.0162	.0340	.0638	5	.0204	.0424	.0798
Oil meal	1	.0126	.0186	.0566	1	.0126	.0186	.0566	1	.0126	.0186	.0566	1	.0126	.0186	.0566
Total	----	.4654	.2190	.5216	----	.3588	.1920	.4484	----	.3546	.1836	.4324	----	.4378	.2132	.5054
Less refuse	----	.0030	.0004	.0022	----	.0048	.0006	.0030	----	.0072	.0010	.0088	----	.0144	.0018	.0018
Total consumed	----	.4624	.2186	.5194	----	.3540	.1914	.4454	----	.3474	.1826	.4236	----	.4234	.2114	.5036

SECOND DIGESTION TRIAL

Alfalfa	14	0.3164	0.1344	0.3724	10	0.2260	0.0960	0.2660	10	0.2260	0.0960	0.2660	12	0.2712	0.1152	0.3192
Silage	30	.0900	.0438	.1164	25	.0750	.0364	.0970	25	.0750	.0364	.0970	30	.0900	.0438	.1164
Oats	4	.0218	.0456	.0806	4	.0218	.0456	.0806	4	.0218	.0456	.0806	4	.0218	.0456	.0806
Corn	5	.0204	.0424	.0798	5	.0204	.0424	.0798	4	.0162	.0340	.0638	5	.0204	.0424	.0798
Oil meal	1	.0126	.0186	.0566	1	.0126	.0186	.0566	1	.0126	.0186	.0566	1	.0126	.0186	.0566
Total	----	.4612	.2848	.7058	----	.3558	.2390	.5800	----	.3516	.2306	.5640	----	.4160	.2656	.6526

TABLE IV.—*Analysis of urine* [a] *(both digestion trials)*

Animal	First digestion trial			Second digestion trial		
	K_2O	P_2O_5	N	K_2O	P_2O_5	N
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Cow No. 1	1.050	0.003	0.370	1.470	0.004	1.147
Cow No. 2	1.460	.004	.663	1.310	.004	1.120
Cow No. 3	1.660	.005	.794	1.760	.006	1.532
Cow No. 4	1.490	.003	.734	1.530	.004	1.286

[a] During both periods the urine and feces were carefully and separately collected, and separate analyses were made of composite samples of each collection.

TABLE V.—*Analysis of feces* [a] *(both digestion trials)*

Animal	First digestion trial			Second digestion trial		
	K_2O	P_2O_5	N	K_2O	P_2O_5	N
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Cow No. 1	0.050	0.140	0.340	0.080	0.140	0.386
Cow No. 2	.050	.170	.318	.090	.160	.348
Cow No. 3	.050	.160	.312	.070	.160	.347
Cow No. 4	.070	.120	.280	.060	.130	.342

[a] During both periods the urine and feces were carefully and separately collected, and separate analyses were made of composite samples of each collection.

TABLE VI.—*The daily urine and feces, and the constants contained therein*

FIRST DIGESTION TRIAL

Animal	Urine per day	Feces per day	K_2O per day			P_2O_5 per day			N per day		
			In urine	In feces	Total	In urine	In feces	Total	In urine	In feces	Total
	Pounds	*Pounds*	*Pound*	*Pound*	*Pound*	*Pound*	*Pound*	*Pound*	*Pound*	*Pound*	*Pound*
Cow No. 1	23.90	67.59	0.2510	0.0338	0.2848	0.0008	0.0946	0.0954	0.0884	0.2298	0.3182
Cow No. 2	21.30	56.40	.3110	.0280	.3390	.0008	.0958	.0966	.1412	.1794	.3206
Cow No. 3	14.45	56.48	.2398	.0282	.2680	.0008	.0902	.0910	.1148	.1774	.2922
Cow No. 4	12.85	77.75	.1914	.0547	.2461	.0004	.0932	.0936	.0944	.2174	.3118

SECOND DIGESTION TRIAL

Cow No. 1	22.40	65.50	0.3292	0.0524	0.3816	0.0010	0.0916	0.0926	0.2568	.2528	0.5096
Cow No. 2	21.95	52.90	.2874	.0476	.3350	.0008	.0846	.0854	.2458	.1840	.4298
Cow No. 3	14.90	46.80	.2622	.0328	.2950	.0008	.0748	.0756	.2282	.1624	.3906
Cow No. 4	19.00	58.80	.2906	.0352	.3258	.0008	.0764	.0772	.2444	.2010	.4454

Table VII, based on all the data in the preceding tables, is given to facilitate a comparison between the daily milk production and the quantities of potash, phosphorus, and nitrogen recovered daily in the liquid and solid manures.

TABLE VII.—*Daily milk production, and pounds of potash, phosphorus, and nitrogen recovered*

FIRST DIGESTION TRIAL

Animal	Milk produced	K_2O			P_2O_5			N		
		Consumed	Voided	Recovered	Consumed	Voided	Recovered	Consumed	Voided	Recovered
	Pounds	*Pound*	*Pound*	*Per cent*	*Pound*	*Pound*	*Per cent*	*Pound*	*Pound*	*Per cent*
Cow No. 1	24.16	0.4624	0.2848	61.59	0.2186	0.0954	43.64	0.5194	0.3182	61.26
Cow No. 2	22.66	.3540	.3390	95.76	.1914	.0966	50.46	.4454	.3206	71.98
Cow No. 3	18.44	.3474	.2680	77.14	.1826	.0910	49.83	.4236	.2922	68.98
Cow No. 4	25.42	.4234	.2458	58.05	.2114	.0936	44.27	.5036	.3118	61.91

SECOND DIGESTION TRIAL

Cow No. 1	20.74	0.4612	0.3816	82.74	0.2848	0.0926	32.51	0.7058	0.5096	72.20
Cow No. 2	23.36	.3558	.3350	94.15	.2390	.0854	35.73	.5800	.4298	74.10
Cow No. 3	18.92	.3516	.2950	83.91	.2306	.0756	32.78	.5640	.3906	69.25
Cow No. 4	23.58	.4160	.3258	78.31	.2656	.0772	29.06	.6526	.4454	68.25

CONCLUSION

During the first of the two trials the amount of potash recovered in the manure was between 58 and 96 per cent of the total fed; the recovery of phosphates ranged between 44 and 50 per cent of the total fed; and the recovery of nitrogen ranged from 61 to 72 per cent. In the second trial the ranges were, for potash, 78 to 94 per cent; for phosphates, 29 to 36 per cent; for nitrogen, 68 to 74 per cent. From these results it seems fair to presume that the dairyman may expect to recover to his land approximately four-fifths of the potash, two-fifths of the phosphates, and two-thirds of the nitrogen fed to lactating cows. This is a saving of feed values that is by no means insignificant.

Contrary to such information as the writers have been able to gain from the literature, they have found that, after taking all precautions for accuracy of analysis, blanks having been run on the reagents used, there is an appreciable and measurable quantity of phosphorus voided in the urine.

SUMMARY

Recognizing that the drawing of general conclusions on the basis of these limited data may not be warranted, the writers submit certain inferences which are apparently warranted; but these inferences are, of course, subject to amendment by further investigation and study.

The quantity of the different constants—nitrogen, phosphorus, and potassium—here examined varies quite markedly. There is consistently more of the potash and nitrogen recoverable in feces and urine than of phosphorus. This fact, however, by no means new to the literature, is but merely confirmatory.

The quantities of the nitrogen, phosphorus, and potassium vary strikingly with the type of animal. In this experiment the highest values returned to the soil through the voided material were returned by the Ayrshire, which also maintained a high milk production at the same time.

The quantities varied with the daily milk production. In a very general way, the recovery of potash and nitrogen was in inverse relation to milk production, while the phosphorus recovery paralleled the production of milk. But the last-named constant is seen to be less influenced by the amount of milk produced than the other two.

The above-suggested relation between milk production and the potash and nitrogen voided is more apparent in the instances wherein the daily milk production varies widely.

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON CEREALS. IV. ON THE PRESENCE OF AMINO ACIDS AND POLYPEPTIDES IN THE UNGERMINATED RYE KERNEL [1]

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INTRODUCTION

In continuing the work reported in previous papers on wheat (*Triticum vulgare*) (*10*),[2] oats (*Avena sativa*) (*11*), and corn (*Zea mais*) (*12*), it was found further that the ungerminated kernel of rye (*Secale cereale*) also contains polypeptides and amino acids. A careful perusal of the literature reveals no report of the presence of these compounds in the ungerminated rye kernel.

Einhof (*3*) was the first to report the presence in rye seed of albumin and of a protein soluble in alcohol. The occurrence of an alcohol-soluble protein was also reported by Heldt (*5*), who gave a complete analysis of it. According to Von Bibra (*1*), the albumin, "casein," and the alcohol-soluble protein in the rye kernel are identical with those in the wheat kernel, while according to Ritthausen (*17*) the rye kernel contains three proteins, namely, albumin, an alcohol-soluble protein (mucedin), and one soluble in dilute potassium hydroxide (gluten-casein).

From the work of Osborne (*13*), we now know definitely that the rye kernel contains five proteins, in the following proportions:

	Per cent
Albumin (leucosin)	0.43
Globulin (edestin) and proteose	1.76
Gliadin	4.00
Glutelin	2.44
Total	8.63

According to this investigator (*14*, *15*, *p. 79*, and *16*), wheat and rye yield similar quantities of albumins and globulins, which seem to be identical, and also the same quantity of gliadin.

Of the nonproteins, Schulze (*18*) reports that the seed of rye contains on the average 0.195 per cent, calculated on the basis of the oven-dried seed, or 9.4 per cent, calculated on its total nitrogen. Schulze concludes from his observations that these figures are more or less fluctuating, depending on the state of ripeness of the seed, the percentage of nonproteins being ordinarily higher in unripe seed. According to Schulze (*19*), there occurs in the seed of rye a nitrogenous phosphatide which contains about 2 per cent of phosphorus, while according to Czapek (*2*, *p. 157*), the seed contains 0.57 per cent of lecithin when calculated to its oven-dried state. As mentioned above, the presence of polypeptides and amino acids in the ungerminated rye kernel is shown for the first time in this paper, so far as the writers are aware.

EXPERIMENTAL DATA

For this work North Dakota No. 959, Reg. Rosen, and Von Rümker varieties of rye were used. The samples were first dried in an electric oven at about 50° C. for one to two days, then ground in a buhr mill, and passed through a 60-mesh sieve.

METHODS USED

The total and the protein nitrogen were determined according to Kjeldahl's and Stutzer's methods, respectively. The amino and the peptide nitrogen were estimated by the formol-titration method (*6*, *20*) as used by the senior author and described in previous publications (*7*, *8*, *9*, *10*, *11*, *12*). Some details of these and other methods will be described subsequently. General information concerning the chemical composition of the three varieties of rye will be found in Table I.

In Table I it will be noticed that the percentage of total nitrogen is highest in North Dakota No. 959, lowest in Reg. Rosen, while the figure for Von Rümker is between the two. The same relationship holds good for the non-

[1] Received for publication Aug. 6, 1924; issued July, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 992.

protein nitrogen and for the protein nitrogen when the latter is referred to the oven-dried substance. The reverse, however, is true when the protein nitrogen is referred to the total nitrogen. As to the ash content, Von Rümker rye is characterized by the highest percentage of ash, followed by Reg. Rosen and North Dakota No. 959 in the order named.

Inasmuch as both aqueous and alcoholic extracts were used for this work, we have determined the proportion of nitrogen that could be extracted by both solvents under certain conditions.

Ordinarily 25-gram portions of flour were treated with 500 c. c. of boiling-hot ammonia-free water and kept on the water bath for 30 minutes and then filtered or centrifuged. The solid resi-

TABLE I.—*Proportion of ash and of total, of protein, and of nonprotein nitrogen in the ungerminated rye kernel*

Variety of rye	Where and when grown	Total nitrogen, oven-dried rye	Protein nitrogen		Nonprotein nitrogen		Ash oven-dried rye
			Oven-dried rye	Total nitrogen	Oven-dried rye	Total nitrogen	
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
North Dakota No. 959 rye	Grown at Dickinson, N. Dak., in 1922.	2.35	2.01	83.40	0.40	16.60	1.79
		2.41	1.99	82.77	.42	17.23	1.79
		2.42	2.06	85.50	.35	14.50	1.78
		2.45	------	------	------	------	1.80
		2.43	------	------	------	------	1.78
		2.43	------	------	------	------	------
		2.41	------	------	------	------	------
Average		2.41	2.02	83.89	.39	16.11	1.79
Von Rümker rye, C. I. 133.	Grown at —— in 1923	1.86	1.60	87.02	.24	12.98	2.13
		1.83	1.55	84.38	.29	15.62	2.13
		1.85	1.58	85.79	.26	14.21	2.06
		1.83	------	------	------	------	2.10
Average		1.84	1.58	85.73	.26	14.27	2.11
Reg. Rosen rye No. R 22, 198.	Grown at Parma, Mich., in 1922.	1.65	1.48	89.71	.17	10.29	2.04
		1.62	1.53	92.73	.12	7.27	2.02
		1.64	1.52	92.12	.13	7.88	2.04
		1.66	------	------	------	------	2.04
		1.67	------	------	------	------	------
		1.65	------	------	------	------	------
		1.67	------	------	------	------	------
Average		1.65	1.51	91.52	.14	8.48	2.04

TABLE II.—*Proportion of nitrogen extracted by water and alcohol*

Variety of rye	Nitrogen extracted by water		Nitrogen extracted by 92 per cent alcohol	
	Milligrams	In per cent of oven-dried flour	Milligrams	In per cent of oven-dried flour
North Dakota No. 959 Rye	50.9684	0.55	56.4043	0.152
	50.9123	.55	56.4183	.152
Average	------	.55	------	.152
Von Rümker Rye, C. I. 133	44.9581	.49	58.2395	.158
	45.8267	.50	58.9121	.160
Average	------	.50	------	.159
Reg. Rosen Rye No. R 22198	44.6499	.48	56.2782	.152
	43.8093	.47	55.7178	.150
Average	------	.48	------	.151

dues were treated once more in the same manner. The filtrates were then made up to 1 liter, of which 400 c. c. portions were oxidized according to Kjeldahl's method. In the case of alcohol, 100-gram portions of flour were extracted with boiling 92 per cent alcohol, the extracts being separated by suction on Buchner funnels from the solid residues, which were extracted once more in like manner. Filtrates and washings were now made up to 1,000 c. c., in 400 c. c. portions, from which the alcohol was first removed by distillation and the nitrogen then estimated in the residue according to the Kjeldahl method. The results are recorded in Table II.

Reference to Table II shows that both the aqueous and alcoholic extractions were quite uniform, but that water extracted about three times as much nitrogen as did the alcohol. This is perhaps due to a greater proportion of protein matter taken up by the water.

In order to demonstrate the presence of amino acids and polypeptides in the rye kernel a series of flasks containing definite quantities of flour were treated with boiling-hot ammonia-free water, which was followed by digestion on the steam bath for 15 to 30 minutes. The extract was now separated by filtration or centrifugalization from the solid residue, which was treated once more in like manner. The extracts were then concentrated under reduced pressure, the precipitates formed (proteins, bran, etc.) removed by centrifugalization, the supernatant liquid evaporated to dryness, extracted with 85 per cent alcohol, filtered, the alcohol distilled off, and finally evaporated to dryness. The dry residue, a dry yellow sirup, was taken up with hot water, filtered, cooled, and made up to 100 c. c. (or its multiple), of which two portions of 10 c. c. each were oxidized according to Kjeldahl's method. To the remaining solution sulphuric acid was added to a concentration of 5 per cent, which was followed by treatment with a phosphotungstic acid solution containing 5 grams of sulphuric acid and 20 grams of phosphotungstic acid per 100 c. c., using but a slight excess of the precipitant. After 24 hours the heavy precipitate was filtered out and thoroughly washed with a solution containing 5 gm. of sulphuric acid and 2.5 gm. of phosphotungstic acid per 100 c. c. The precipitate formed by phosphotungstic acid ordinarily removes any proteins, proteoses, and peptones present. That it also contained diamino acids was shown in the following manner: The precipitate was treated with barium hydroxide, the excess of which was removed with carbon dioxide, the whole was filtered off with suction, and the remaining cake thoroughly washed with hot water. Filtrate and washings were evaporated in vacuo practically to dryness, taken up with a few cubic centimeters of water, and filtered. The filtrate, which had a light-yellow color, gave the following reactions:

(1) Phosphotungstic acid gave at once a heavy white precipitate.
(2) Phosphomolybdic acid gave immediately a yellow precipitate.
(3) Silver nitrate gave a grayish precipitate soluble in excess of ammonia.
(4) Addition of neutralized formaldehyde caused the solution to become acid.
(5) Mercuric chloride gave a grayish flocculent precipitate.

The filtrate from the phosphotungstic precipitate was freed from sulphuric and phosphotungstic acids by treatment with calcium hydroxide to slight acidity, then with barium hydroxide to alkalinity, the excess of barium hydroxide being removed with carbon dioxide. The whole was now brought to a boil and filtered, the solid residue being extracted once more with hot ammonia-free water. The filtrate and washings were then concentrated under reduced pressure and made up to 100 c. c., of which two portions of 20 c. c. each were oxidized by the Kjeldahl method, while 50 c. c. were (on being freed from carbon dioxide, phosphoric acid, and coloring matter) formol-titrated, which gave the nitrogen of monoamino acids.

The presence of polypeptides was demonstrated as follows: The flour extract and the phosphotungstic acid precipitate were obtained exactly in the manner just described. Equally, the filtrate from the phosphotungstic precipitate was freed from sulfuric and phosphotungstic acids and finally concentrated in vacuo to 100 c. c., as outlined above. In two 10 c. c. portions the nitrogen was estimated according to the Kjedahl method. To the remaining 80 c. c. hydrochloric acid was added to a concentration of 20 per cent and hydrolyzed under a reflux condenser for 12 hours, in accordance with the observations of Fischer (*4*). The hydrolysate was now evaporated on the water bath to dryness, taken up with hot water, and distilled with magnesium oxide in order to remove the ammonia. The residue was then thoroughly extracted with hot water (which was previously freed from ammonia by long boiling), evaporated, and made up to 100 c. c., of which two portions of 20 c. c. each

were oxidized according to Kjeldahl's method, while 50 c. c. of the remaining solution were used for formol-titration.

For the estimation of the nitrogen of acid amides the extraction of the flour as well as the precipitation with phosphotungstic acid, etc., were effected as outlined in the case of the polypeptides, but the solution to which hydrochloric acid had been added to a concentration of 20 per cent was hydrolyzed for only 30 minutes. It was now evaporated to dryness, and the residue, which was taken up with hot water, was distilled with cream of magnesia, the ammonia being received in an Erlenmeyer flask containing N/10 H_2SO_4. The results are summarized in Table III.

Examination of Table III shows that when the results are referred to the water-soluble nitrogen the monoamino nitrogen is highest in Reg. Rosen (20.62 per cent), slightly lower in Von Rümker (20.10 per cent), and considerably lower in North Dakota No. 959 (13.56 per cent). As to the peptide nitrogen, it is highest in Von Rümker (30.96 per cent), somewhat lower in North Dakota No. 959 (29.36 per cent), and lowest in Reg. Rosen (14.55 per cent). Concerning the nitrogen of acid amides, it is highest in Von Rümker (18.50 per cent), lowest in Reg. Rosen (14.28 per cent), while the figure for North Dakota No. 959 is between the two. The outstanding feature of the results presented in Table III is the fact that the three varieties investigated contain not inconsiderable quantities of amino acids and polypeptides in their ungerminated kernels.

TABLE III.—*Distribution of the nonprotein nitrogen in the ungerminated rye kernel*

Variety of rye	Nitrogen of acid amids	Nitrogen of amino acids	Peptide nitrogen
Percentage of the water-soluble nitrogen of the rye kernel:	*Per cent*	*Per cent*	*Per cent*
North Dakota No. 959 Rye	{	13.96	29.53
	{	13.16	29.19
Average	16.32	13.56	29.36
Von Rümker Rye C. I. 133	{	19.71	30.64
	{	20.49	31.27
Average	18.50	20.10	30.96
Reg. Rosen Rye No. R 22198	{	20.77	14.80
	{	20.47	14.29
Average	14.28	20.62	14.55
Percentage of the total nitrogen of the rye kernel:			
North Dakota No. 959 Rye	{	3.18	6.73
	{	3.00	6.66
Average	3.72	3.09	6.69
Von Rümker Rye C. I. 133	{	5.28	8.21
	{	5.49	8.38
Average	4.96	5.39	8.30
Reg. Rosen Rye No. R 22198	{	6.01	4.29
	{	5.93	4.14
Average	4.14	5.97	4.21
Percentage of the oven-dried rye kernel:			
North Dakota No. 959 Rye	{	.077	.162
	{	.072	.161
Average	.090	.075	.162
Von Rümker Rye C. I. 133	{	.099	.153
	{	.103	.156
Average	.093	.101	.155
Reg. Rosen Rye No. R 22198	{	.100	.071
	{	.098	.069
Average	.069	.099	.070

It seemed of certain interest to extract the amino acids of the flour samples *directly* with alcohol instead of indirectly, as was done in the experiments described above. Hence definite quantities of flour were extracted with boiling 92 per cent alcohol, the extracts being separated by suction on a Büchner funnel from the residues, which were once more extracted in the same manner. The alcohol was removed from the combined extracts by distillation in a vacuum. The residues were alternately treated with ether (to remove fat) and hot water, and the ethereal and aqueous extracts separated in a separatory funnel. The aqueous extract was treated several times with small quantities of ether, while the ethereal extract was treated repeatedly with small quantities of water. The combined aqueous extracts, which contained the amino acids, were kept on the water bath for some time in order to remove some ether present, whereupon they were filtered. The solution was now slightly acidified with acetic acid and treated with 10 per cent tannic acid. The precipitate formed (chiefly protein) was filtered off, the filtrate was concentrated and made up to a definite volume, which was then treated with phosphotungstic acid in the manner already described. The filtrate from the phosphotungstic-acid precipitates was then treated with calcium hydroxide to slight acidity, then with barium hydroxide to alkalinity, saturated with carbon dioxide, boiled, filtered, the filtrate being concentrated under diminished pressure to 100 c. c. in two 20 c. c. portions, of which nitrogen was estimated according to Kjeldahl's method, while 50 c. c. were used for formol-titration. In this manner it was found that North Dakota No. 959 contains 19.95 per cent, 20.90 per cent, on the average 20.43 per cent, of monoamino nitrogen; while Reg. Rosen contains 35.51 per cent, 34.63 per cent, on the average 35.07 per cent, of monoamino nitrogen, when calculated on the basis of the *alcohol-soluble* nitrogen. These results, while not strictly comparable with those obtained with the aqueous extract, confirm in a general way the conclusion drawn from Table III that the ungerminated kernels of the rye varieties contain appreciable quantities of free amino acids.

TABLE IV.—*Distribution of the nonprotein nitrogen in the ungerminated kernel of wheat, oats, and corn*

Variety of cereal	Nitrogen of acid amides	Nitrogen of amino acids	Peptide nitrogen
Percentage of oven-dried *wheat* kernel:	*Per cent*	*Per cent*	*Per cent*
Fultz	0.026	0.032	0.084
Kanred	.053	.066	.111
Kubanka	.052	.041	.155
Marquis	.058	.054	.151
Percentage of total nitrogen of *wheat* kernel:			
Fultz	1.46	1.77	4.67
Kanred	1.88	2.34	3.89
Kubanka	1.72	1.35	3.13
Marquis	1.91	1.77	4.98
Percentage of water-soluble nitrogen of *wheat* kernel:			
Fultz	8.76	10.66	28.09
Kanred	12.99	16.25	26.86
Kubanka	12.61	9.91	37.76
Marquis	12.33	11.46	32.20
Percentage of oven-dried *oat* kernel:			
Swedish Select	.051	.064	.106
Victory	.029	.040	.073
Iowar	.046	.057	.084
Winter Turf	.027	.025	.032
Percentage of total nitrogen of *oat* kernel:			
Swedish Select	1.95	2.48	4.10
Victory	1.45	2.02	3.63
Iowar	1.93	2.35	3.49
Winter Turf	1.80	1.65	2.15
Percentage of water-soluble nitrogen of *oat* kernel:			
Swedish Select	13.69	17.39	28.71
Victory	10.86	15.08	27.16
Iowar	12.18	14.87	22.03
Winter Turf	15.01	13.79	17.88
Percentage of oven-dried *maize* kernel:			
Four County Corn	.032	.045	.069
U. S. Selection No. 77	.019	.040	.050
Hall Gold Nugget Selection No. 193	.021	.051	.036
Percentage of total nitrogen of *maize* kernel:			
Four County Corn	1.88	2.65	4.06
U. S. Sleection No. 77	1.19	2.52	3.14
Hall Gold Nugget Selection No. 193	1.44	3.49	2.47
Percentage of water-soluble nitrogen of *maize* kernel:			
Four County Corn	15.61	21.97	34.07
U. S. Selection No. 77	12.18	26.67	32.79
Hall Gold Nugget Selection No. 193	11.20	26.95	19.17

For convenient comparison the results obtained with the various cereals are presented in Table IV.

Although the figures in Tables III and IV are somewhat fluctuating, they indicate that the nitrogen percentage of amino acids is highest in the kernel of corn, lowest in that of wheat, and between the two for the rye and oat kernel. As to the percentage of peptide nitrogen, it is highest in the wheat kernel, followed by the kernels of maize, rye, and oats in the order named. The nitrogen percentage of acid amides may be said to be highest in the rye kernel and lowest in the wheat kernel, while the nitrogen percentage of the oat and maize kernel is between the two.

It may not be amiss here to mention that wheat and rye, each of which contains five proteins, namely, albumin, globulin, proteose, glutenin, and gliadin, thus showing a striking similarity, also display some similarity with regard to their nonproteins.

SUMMARY

Polypeptides and amino acids have been shown in this paper to occur in the ungerminated rye kernel.

The proportions of amino nitrogen in the varieties North Dakota No. 959, Von Rümker, and Reg. Rosen have been found to be, respectively, 3.09, 5.39, and 5.97 per cent, calculated on the basis of the total nitrogen; and 0.075, 0.101, and 0.099 per cent, calculated on the basis of the oven-dried kernel.

The percentages of peptide nitrogen are 6.69 for North Dakota No. 959, 8.30 for Von Rümker, and 4.21 for Reg. Rosen, calculated on the total nitrogen; and 0.162 for North Dakota No. 959, 0.155 for Von Rümker, and 0.070 for Reg. Rosen, calculated on the oven-dried kernel.

The nitrogen of acid amides in the varieties North Dakota No. 959, Von Rümker, and Reg. Rosen makes up, respectively, 3.72, 4.96, and 4.14 per cent, calculated to the total nitrogen; and 0.090, 0.093, and 0.069 per cent, calculated to the oven-dried kernel.

Inasmuch as we have shown that the ungerminated kernel of wheat, oats, maize, and rye contains polypeptides and amino acids, it seems safe to state that ungerminated kernels of the other cereals, such as rice, sorghum etc., also undoubtedly contain these substances.

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FACTORS AFFECTING REPRODUCTION OF ENGELMANN SPRUCE [1]

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INTRODUCTION

With the purpose of obtaining much-needed information regarding the laws governing reproduction of Engelmann spruce (*Picea engelmanni*) in the northern Rocky Mountain region, studies were undertaken during the summers of 1920 and 1921 covering representative tracts of cut-over and burned-over land in the spruce type, as well as additional reproducing areas in the natural forest. The areas studied were located in western Montana, in the Blackfeet National Forest. While the data obtained must be regarded as incomplete in many respects, they are of considerable value in view of the great lack up to the present time of sufficient controlled examinations of spruce reproduction, and the far too theoretical conceptions of the requirements for the establishment of the species that have prevailed. Current timber sales on the national forests, as well as the increase in cutting in spruce stands that is now in sight, make imperative a more definite understanding of these requirements, and bring about an urgent demand for data that may be made the basis of timber marking, slash disposal, and selection of logging equipment. As at least a partial response to this demand, the following data and conclusions are submitted.

SEED PRODUCTION

Engelmann spruce is a heavy seed producer. Good crops of cones are produced at intervals of three to four years, while in intermediate years individual trees yield heavy crops of seed. The cones are produced in quantities at the tops of the tree crowns and in the northern Rockies the seed ripens about September 15. Southerly or northerly exposure will hasten or retard ripening about a week. After October 1, seed begin to shed.

The number of seeds average 125,000 per pound for Colorado, 122,400 for Utah, and 190,000 for Montana (3-year average). In past tests, from 0.8 to 1.2 pounds of Montana seed have been extracted from 1 bushel of cones, or at an average rate of 190,000 seeds to the bushel. No data are available on the number of bushels of cones to the acre that a stand of mature trees will produce. It is probable, however, that not less than 3 or 4 bushels per acre are borne by the trees in a good seed year. The quantity of seed thus showered upon the forest floor is very great.

Germination varies widely. Tests at the Savenac Nursery, Lolo National Forest, Montana, indicate that a germination of 50 to 60 per cent is possible. Nevertheless, spruce seedlings are few in number under most natural conditions. Why this should be generally true is a question that can be answered here in part at least. The answer will also serve to indicate the means of obtaining reproduction in logged-off areas.

REPRODUCTION STUDIES

Many of the factors which influence the survival of spruce seedlings are to be seen in the life histories of spruce stands under natural conditions. Although the course of renewal of such stands varies with the zonal position in the spruce type, a general uniformity of reaction to physical conditions is evident. Within the type local variations occur, due to soil, abrupt slopes, or exposure, but these will not affect the conclusions for the type generally.

BURNED-OVER AREAS—UPPER ALTITUDINAL ZONE

A field study of reproduction in the upper altitudinal spruce zone was made

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in the vicinity of Moose Lake in the Big Creek drainage of the Blackfeet National Forest. In 1910 a forest fire swept across a glacial valley below Moose Lake in a northeasterly direction. The site of this burn on both northerly and southerly slopes around Moose Lake furnished an opportunity to study the extent to which spruce and associate species come in on burned surfaces.

The altitude of the valley is 5,000 to 6,500 feet but the study was confined within a range of 5,000 to 6,000 feet. Up to the 6,000-foot contour the stand 12 inches and over in diameter breast high consists of 63 per cent Engelmann spruce and 37 per cent alpine fir (*Abies lasiocarpa*), as shown by the reconnaissance survey for a representative section in this locality. In board foot volume, however, the proportion is 80 per cent for the spruce and 20 per cent for the fir, accounted for by the difference in size of the two species, which yielded, respectively, 7.5 and 16.5 logs per thousand board feet.

On the southerly exposures lodgepole pine (*Pinus contorta*) formed a small percentage of the mixture.

Altitude affects the mixture of the stand noticeably. At about the 6,000-foot contour the spruce ceases to form any considerable portion of the mixture (pl. 1). The alpine fir, however, continues on to higher altitudes and the whitebark pine (*Pinus albicaulis*) replaces the spruce. The stands above 6,000 feet are purely protection forests and are disregarded for the purpose of this report.

The number of surviving seedlings may be considered a reasonable indicator of site conditions. This conclusion is strengthened by an examination of the vegetative cover, of which only the dominant species of plants and shrubs are given below. The thin vegetative cover on the upper southwest slope and the presence of the more drought-resistant species indicate the severity of the site:

Southwest exposure; vegetation (dominant species in order of occurrence):
- Upper slope (13 chains); vegetation very thin—20 to 60 per cent bare soil; soil shaley—
  - Vaccinium membranaceum (?).
  - Xerophyllum tenax.
  - Salix sp.
  - Chamaenerion angustifolium.
  - Amelanchier alnifolia.
  - Acer sp. scattered clumps.
  - Alnus sp.
- Middle slope (13 chains); vegetation thin—10 to 70 per cent bare soil; moisture at lower end of strip—
  - Alnus sp.
  - Salix sp.
  - Chamaenerion angustifolium.
  - Xerophyllum tenax.
  - Pachystima myrsinites.
  - Aquilegia sp.

Southwest exposure—Continued.
- Middle slope—Continued.
  - Acer sp.
  - Populus tremuloides.
- Lower slope (13 chains); vegetation cover thin to dense—
  - Rubus parviflorus.
  - Menziesia glabella.
  - Pachystima myrsinites,
  - Xerophyllum tenax.
  - Vaccinium sp.
  - Acer sp.
  - Salix sp.
  - Ribes sp.

Northeast exposure; vegetation (dominant species in order of occurrence):
- Upper slope (3 chains); vegetation dense to thin—
  - Rubus parviflorus.
  - Menziesia glabella.
  - Chamaenerion angustifolium.
  - Alnus sp.
  - Arnica latifolia.
  - Moss, covering mineral soil.
- Lower slope (8 chains); vegetation very dense, waist-high—
  - Menziesia glabella.
  - Lycopods and moss (under brush cover).
  - Ribes sp.
  - Alnus sp.
  - Vaccinium sp.
  - Arnica latifolia.
  - Acer sp.

As a means of studying the burned area a 55-chain transect was run from the top of one ridge down a southwest slope, across the valley, and up the opposite northeast slope to the top of the opposite ridge. In addition, random sample plots were located at advantageous points.

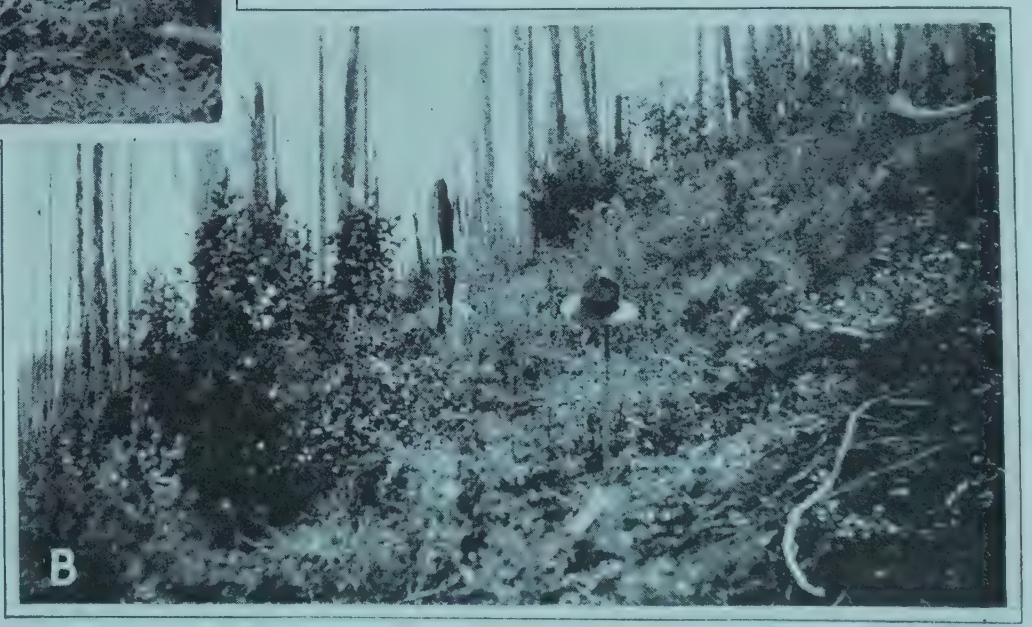
The transect run in this manner enabled a very satisfactory study of both slopes, and Table I discloses an interesting set of conditions on opposite exposures within the same type. The varying number of seedlings to the acre found on each division of the respective slopes is an index of the complex responsible for the restocking. More seedlings by a large ratio occur on the protected northeastern and lower southwestern slopes. The more moist condition favoring survival is at the same time responsible for a larger seed supply. On these sites, too, a few mature trees escaped the fire; whereas on the upper southwest exposure the drier conditions favored a harder burn and the killing of all the trees (pl. 2, A and B).

Despite the absence of mature living trees within a chain on each side of the transect on the southwest slope, the presence of seedlings less than 5 years old indicates the continuous reseeding of the light-seeded species by wind-blown seed. Scattered trees, particularly on ridges, are doubtless the sources of these seed.

Thus the interruption of the natural course of plant succession by the forest fire has had different effects on the two exposures. As presumably the process of reseeding gave approximately equal opportunity for reproduction over the

The upper limit of the spruce type, Northwestern Montana. Alpine fir and whitebark pine compose the forest cover at altitudes above this

A.—Although no Engelmann spruce seed trees remained alive within a quarter of a mile of this spot, three spruce seedlings are visible, two near the hat and one near the hatchet. This is the top of the ridge near the starting point of the transect. Over the break of the hill to the southwest no spruce seedlings were found. (Moose Lake area)



B.—A very heavy stand of Engelmann spruce and alpine fir seedlings has come in here, on the northeast exposure. Engelmann spruce seedlings were the more numerous of the two on the drier portions of this slope

TABLE I.—*Composition of transect on Moose Lake area, 0.1 chain wide by 55 chains long*

Exposure and slope	Gradient	Seedlings per acre, by species and age classes								Total seedlings per acre	Trees in original stand [b]
		Engelmann spruce		Alpine fir		Lodgepole pine		Miscellaneous species [a]			
		Years		Years		Years		Years			
		0 to 5	6 to 10	0 to 5	6 to 10	0 to 5	6 to 10	0 to 5	6 to 10		
Southwest exposure:	*Per cent*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	
Upper slope (13 chains).	10–40	------	8	15	62	------	138	23	------	246	LPP., AF., WBP., DF.
Middle slope (13 chains).	20–40	8	54	62	216	8	185	15	15	563	AF., ES., DF.
Lower slope (13 chains).	10–40	31	562	23	1,380	------	154	------	231	2,381	ES., AF., DF. [c]
Northeast exposure:											
Lower slope (8 chains).	35–75	863	2,840	288	2,300	------	12	------	------	6,303	ES., AF. [d]
Upper slope (8 chains).	10–35	450	2,590	288	4,250	------	50	------	37	7,665	ES., AF., WL.

[a] Miscellaneous includes whitebark pine, Douglas fir, and Western larch.
[b] Abbreviations: ES=Engelmann spruce; AF=Alpine fir; WBP=whitebark pine; DF=Douglas fir; WL=Western larch; LPP=lodgepole pine.
[c] Engelmann spruce 14 and 36 inches d. b. h., still alive.
[d] Scattering trees of alpine fir and Engelmann spruce still alive.

entire area, it may be concluded that reproduction of Engelmann spruce can be assured on burned-over areas in the spruce type proper where the soil is favorable to germination and survival and a supply of seed is present. On the southerly exposure above the lower slope, lodgepole pine will first take possession of the site and for a considerable period will be the predominant species. In time the spruce and alpine fir will increase in ratio and, in at least a portion of this type, will eliminate the temporary lodgepole pine. On the northerly exposure, however, a stand of Engelmann spruce and alpine fir will follow immediately.

The importance of taking into full account the influence of exposure on the restocking of spruce stands is here apparent. For example, a selection cut that will give spruce greater advantage over lodgepole pine is indicated for the southerly exposures, while approximately a clean cut will accomplish the same result on the northerly exposures.

IN THE NATURAL FOREST

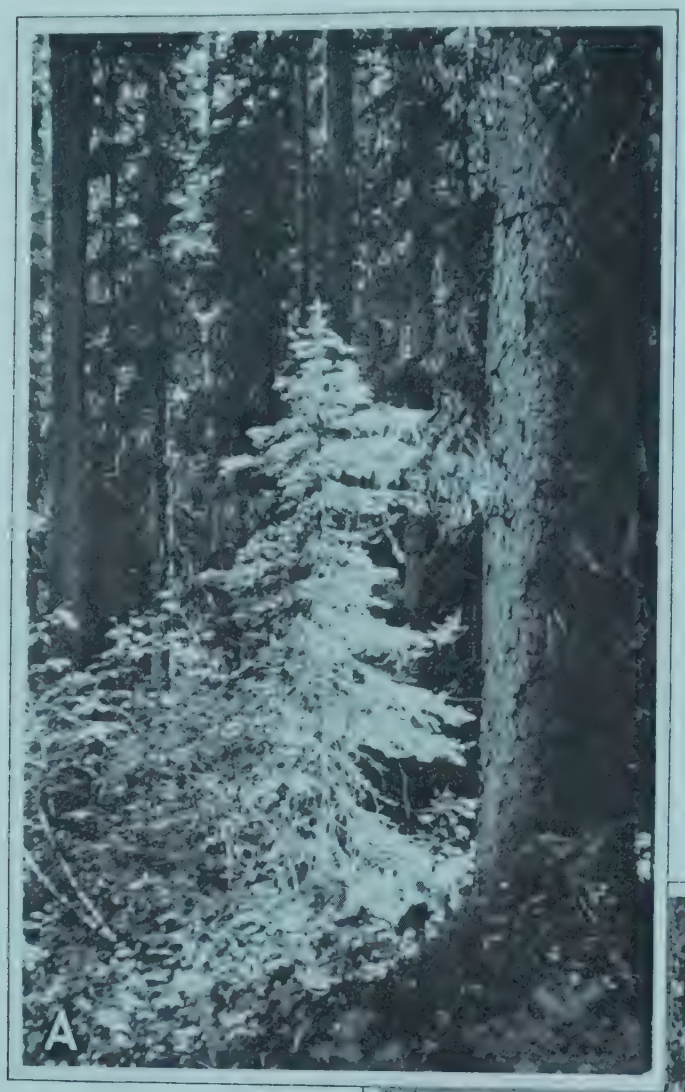
An examination of the course of reproduction in the openings of the natural forest untouched by fire or by man discloses other factors governing the survival of spruce seedlings, which supplement those found to hold true on the burns. Studies for this purpose were made on 11 sample plots in the Blackfeet National Forest, on which conditions surrounding reproduction were very similar to those following a selection cutting in the spruce–fir type.

Both Engelmann spruce and alpine fir are tolerant species, and form forest stands typically suited to selection cutting when the course of plant succession is permitted to continue long enough without disturbance from outside agencies. Consequently these species are found growing up in openings, and causing the old forest to assume a group composition which is indicative of the manner of renewal (pl. 3, A and B).

The natural reproduction per acre generally found in the spruce–alpine fir mixtures is typified in the following figures from the $\frac{1}{16}$-acre sample plots:[2]

Engelmann spruce, up to 2 inches d. b. h.	96
Engelmann spruce, 3 to 8 inches d. b. h.	64
Alpine fir, up to 2 inches d. b. h.	153
Alpine fir, 3 to 8 inches d. b. h.	151

[2] An investigation in Colorado showed that the Engelmann spruce was more numerous than the alpine fir in the reproduction. HODSON, E. R., and FOSTER, J. H. ENGELMANN SPRUCE IN THE ROCKY MOUNTAINS. U. S. Dept. Agr., Forest Serv. Circ 170, 23 p., 1910.



A.—A view of the manner in which the natural forest renews itself. This selection type of renewal is only possible to the tolerant species—in this case Engelmann spruce

B.—The natural spruce forest. On this divide between Canuck Creek and Ruby Creek, in the Pend Oreille National Forest in northern Idaho, the spruce grows up to 36 inches in diameter, yielding from five to seven 16-foot logs to the tree. The old stand is 90 per cent spruce and 10 per cent alpine fir, but the advance growth is just the reverse, with 90 per cent fir and only 10 per cent spruce

The manner in which this advance growth has come in is significant. The forest floor in these stands is covered by a heavy vegetation made up of *Menziesia glabella, M. ferruginea, Vaccinium* sp., *Pachystima myrsinites, Rubus parviflorus*, and *Arnica* sp.

Tag alder and vine maple (*Acer circinatum*) also occur in clumps in the stand. The duff surface, which is well shaded by the stand and the vegetative cover, consists of a heavy mat of undecomposed and semidecomposed needles, leaves, and twigs in which moss appears occasionally.

Seedlings of alpine fir were found in certain numbers on the duff layer but practically no seedlings of spruce (pl. 4). This lack was strikingly noticeable. Spruce seedlings were found, however, on the mounds of upturned root systems of wind-thrown trees and on decaying, moss-covered logs, especially on decaying logs which were covered with a mat of moss under conditions which favor the growth of twin flower (*Linnaea borealis americana*). Seedlings were also found in the bark scale accumulations around the stumps of trees. The occurrence of spruce seedlings on these surfaces in the green timber appears to hold true generally in the spruce type (pl. 5). The complete explanation of this phenomenon will require additional instrumental study. Present findings indicate, however, that this reproduction, while sufficient to perpetuate a forest following a selection cutting—that is, to supply the natural loss—is not sufficient to restock the stand.

Restocking in the northern Rockies runs predominantly to alpine fir in the natural forest. Alpine fir is, however, disposed to defect to a much greater extent than spruce, and never attains the dimensions of the latter. The reconnaissance data for a typical section of 640 acres show the average number of logs per thousand board feet for spruce to be 7.5 and for alpine fir to be 16.5. Thus stocking after selection cutting has the disadvantage of insufficiency and of favoring the less desirable species.

CUT-OVER LAND—MIXED STANDS AT LOWER MARGIN OF TYPE

Old cuttings of timber claims and cuttings along the Great Northern Railway right of way, as well as timber sale cuttings on Nyack and Deerlick Creeks of the Flathead National Forest, Mont., yielded the same conclusions regarding the reproduction of Engelmann spruce as for the natural stands at the higher elevations. The areas studied by sample plots were situated at the lower margin of the spruce type, about Nyack, Mont., on the Great Northern Railway.

TABLE II.—*Detailed observations of occurrence of seedlings in uncut stands or in freshly cut stands at lower margin of type*

Nature of surface	Protection	Engelmann spruce	Alpine fir	Range in age
		Number	*Number*	*Years*
Decaying log covered with moss	Stump shade from south; sarsaparilla, Cornus, and thimbleberry.	4		4 to 9
Decaying stump covered with needles	In shadow of vine maple, fern, and yew	3		5 to 7
Decayed log, now a mound covered with moss and Linnaea.	In shadow of thimbleberry, sarsaparilla, alder, and yew.	42		3 to 10
On humus near the above	In shadow of alder and sarsaparilla	8		2 to 10
10-foot decayed log covered with moss and leaf mold.	In shadow of thimbleberry and sarsaparilla.	32	8	[a] 2 to 10 [b] 2 to 10
Decayed log covered with moss and mold—Linnaea and lycopods.	In shadow of thimbleberry	2	2	[a] 6 to 10 [b] 10
Decayed log covered with moss and leaf mold 1 inch thick.	Shaded only by dense stand overhead	7	4	[a] 7 to 15 [b] 5 to 15
On decayed stump covered with moss and mold.	In shadow of overhead stand	33	3	[a] 3 to 15 [b] 6
On mound of upturned roots 5 feet above ground level; no alpine fir was found here.	In opening of stand; receives direct light about 2 hours in the day.	11		10 to 15
Decayed log stub covered with leaf mold in which twinflower is growing.	In scanty shade of Menziesia and Vaccinium.	9	2	3 to 10

[a] Engelmann spruce. [b] Alpine fir.

An excellent stand of almost pure Engelmann spruce on Big Creek, Blackfeet National Forest. The ground is covered by a dense growth of Menziesia, waist to shoulder high. Under this no reproduction is coming in

Engelmann spruce seedlings growing on the roof of an abandoned cabin. When sufficient protection is given a surface to keep it moist, Engelmann spruce apparently will establish itself and be able to maintain itself for some time almost anywhere

52243—25†——2

Table II presents the general manner of occurrence of Engelmann spruce seedlings in the closed natural stands. These observations do not represent the average condition of restocking for the stand, however, for alpine fir occurs on the forest floor more generally than Engelmann spruce, and in a count of advance growth above one foot in height the alpine fir exceeds in numbers. While the advance growth resulting from this manner of renewal has an important part in the restocking following cutting, it is generally insufficient and must be supplemented by a crop of seedlings, which should be predominantly Engelmann spruce. Few seedlings of any species were discovered on the duff layer underneath a heavy cover of vegetation.

In the opening out of a stand as on these cut-over tracts, where fire has not destroyed the surface material, no seedlings such as are described in Table II were found coming in. The full exposure to sun and wind of decayed logs and upturned root mounds renders these locations unfavorable to the survival of either spruce or alpine fir seedlings. A shortage of soil moisture is the very likely explanation, for on an old tree trunk lying half emersed in a mountain lake were found a large number of seedlings of spruce growing in the decaying and broken-down wood, although in full exposure. While the rôle of surface soil moisture will be more fully treated later, it may be said here that restocking in cut-over stands will not occur as it does in uncut natural stands, and that renewal of the forest will therefore depend on favoring natural reproduction in other ways.

SEEDLING SURVIVAL IN CUT-OVER STANDS

In order to obtain data upon seedlings in cut-over stands two drainage areas were studied, Nyack Creek, in the Blackfeet National Forest, and Ruby Creek in the Pend Oreille National Forest.

THE NYACK TRACTS

The Nyack Creek area includes several kinds of treatment. Of these the homestead cuttings, which in this section are 20 years old or older, did not yield representative data because of the influence of grazing. Some portions of more recent cuttings, however, furnished significant evidence.

The first of these tracts is a cutting made in 1911 on which the slash has been left. The tract is flat; the soil is compact of alluvium over a moraine subsoil. A strip 8 chains long was run across the tract to the edge of a broadcast burn. Over a width of 1 chain on this strip a tally of living trees was made, the area totaling 0.8 acre. On a parallel strip 0.1 chain wide, or 0.08 acre, the advance growth was tallied.

TABLE III.—*Composition of reproduction strip on Nyack area* [a]

Species	Standing living trees (0.8 acre)			Restocking advance growth (0.08 acre)	
	Number	Diameters [b]	Rate per acre	Number	Rate per acre
		Inches			
Engelmann spruce	6	8 to 12	7.5	24	300
Alpine fir	2	8 to 10	2.5	20	250
Others	1	8	1	3	38

[a] The original stand consisted of a mixture of Engelmann spruce, Douglas fir, and western larch. The tract is covered with undisposed-of logging slash which is heavy in places.

[b] Diameter at breast height.

Figures for both tallies are given in Table III. The significant fact that came out in the course of these observations is that none of the seedlings found had become established since logging. Those present were on the ground when the stand was cut. This is corroborated by conditions on a sample plot taken in a different area and summarized in Table IV.

TABLE IV.—*Composition of reproduction plot on the Nyack area*

Species	Living trees (0.25 acre)			Advance growth (0.0625 acre)	
	Number	Diameters [a]	Rate per acre	Number	Rate per acre
		Inches			
Engelmann spruce	7	8 to 14	28	16	256
Alpine fir	0	----------	0	36	576
Western larch	2	10 to 14	8	1	16
Others	6	8 to 14	24	2	32

[a] Diameter at breast height.

The stand represented by Table IV is more dense than that represented by Table III, and there is more advance growth on the ground; but the two sample areas agree in respect to the lack of seedlings since cutting. It was

noticeable that in the open spaces between groups of advance growth no seedlings were present even in the absence of a slash cover, notwithstanding the seeds that were without doubt showered upon the ground, for the spruce trees were bearing cones.

Unfavorable surface soil conditions doubtless account for the absence of seedlings. As skidding had been done in the snow and the soil was not broken, an undecomposed duff layer covered the ground. It is also possible that the thin layer of twigs and leaves heated up so much in the sunlight that tender seedlings were unable to survive the heat. However, this can not be said to explain definitely the absence of seedlings, for more needs to be known of the temperatures which exist in this type of soil cover in the critical summer period.

These two tracts furnishing evidences of unsuccessful reproduction following cutting are "flats." The indication is that spruce stands on flats must not be opened out suddenly if spruce reproduction is expected to return immediately in the succeeding stand. To insure the survival of Engelmann spruce, it is better to avoid this condition of wind-swept, dried-out layers of litter.

Tracts on a northerly slope yielded somewhat different results. Reproduction of Engelmann spruce was present in quantities sufficient for a satisfactory stand. Table V presents the findings on four plots selected as representative of the conditions on the northerly exposures.

TABLE V.—*Average distribution of seedlings per acre, by height classes, northerly exposure, Nyack area*

Species	12 inches high and under	Over 1 foot high
	Number	*Number*
Engelmann spruce	512	496
Western larch	224	256
Douglas fir	256	160
Other species	80	96
Total	1,072	1,008

This restocking came in on mineral soil exposed in clearing the right of way for the Great Northern Railway and on burned ground. It is to be noted that Western larch (*Larix occidentalis*) and Douglas fir (*Pseudotsuga taxifolia*) form an important portion of the new growth. The original stand is reflected only partially in the reproduction, for the stumps show the stand to have consisted of a mixture of larch and fir predominantly, and of lodgepole pine, spruce, and alpine fir to a lesser degree. In the reproduction Engelmann spruce appears to be growing better under the more vigorous larch and lodgepole pine saplings. The soil here is covered by a thin layer of larch needles and twigs, which the spruce has evidently found favorable to survival.

Another tract cleared up some of the perplexing features of the reproduction of the Engelmann spruce. This lies also within the Great Northern Railway right of way.

In building the railroad through this region a strip of forest fully 150 feet wide was cleared. The grade was built up with earth scraped out from the sides, and taken from alternate 20 by 20 foot squares along the track. In this manner the unbroken sod extends practically to the grade, save for the ditch, in alternate tongues 20 feet wide. The excavation squares were scraped down from 2 to 3 feet deep. Thirteen of these squares along the track were carefully examined. In all squares spruce seedlings 20 to 25 years old were found. In a few were lodgepole pine and larch seedlings, but no seedlings were found in the grass sod surrounding these excavations. A count showing the stocking of these squares is as follows:

Engelmann spruce, 12 inches high and under	7
Engelmann spruce, over 1 foot high	101
Lodgepole pine, 12 inches and under	0
Lodgepole pine, over 1 foot high	3
Other species	0

The largest trees were from 20 to 25 years old.

Between and beyond these squares, in which the mineral soil had been exposed, the unbroken sod areas extended to the edge of the right of way. On the grass sod no seedlings were to be found. Thus, of these two distinct kinds of surfaces, spruce was coming in at the rate of over 1,500 per acre on the mineral soil, but in the grass not at all. The seed supply for both types of area was of necessity the same. Both areas were exposed to full light and no shade was cast upon either type during the hot hours of the day. The only variables, therefore, were the soil surface and the moisture content.

It may be concluded that the grass sod in this instance used up the soil moisture so completely as to exclude the tree seedlings. This appears to be the key to the problem of the reproduction of Engelmann spruce. The

species has been shown to establish itself even under full light if favorable conditions obtain. These conditions are sufficient moisture to sustain the seedling over the critical dry season, and a mineral soil or moisture-holding surface.

If surface moisture is the deciding factor, the protection of the overstory serves only to prevent desiccation of the soil below the critical point. But vegetation, even under the canopy of a forest, may create unfavorable surface soil conditions, as was noted in the studies in green timber.

Locally it is possible that the chemical contents of the soil, for example acid conditions in poorly drained sites, may preclude the establishment of the spruce, but such areas comprise so small a percentage of the type that in specifying the guiding silvicultural rules this may be omitted from consideration. Such conditions call for detailed investigations of the factors of site controlling reproduction of the species.

Shade may inhibit the restocking of spruce, but to do so it must be very dense, and only in rare instances will the lack of light preclude restocking. In cut-over areas this factor does not operate, and may be dismissed.

The Nyack tracts therefore indicate that where the surface litter is deep and is not broken up in logging, Engelmann spruce seedlings will not survive; nor will they come in in forest openings on flats or other dry sites. On moist mineral soil, however, and on burned-over ground spruce will establish itself; also where forest litter is thin. Grass cover is a dangerous competitor for moisture, and is liable, if not certain, to eliminate Engelmann spruce reproduction. In general, the decisive factor appears to be surface soil moisture available to the seedling during critical periods of the dry season.

THE RUBY CREEK AREA

The Ruby Creek area was cut over in 1917 and 1918 by a varied series of methods, designed to bring out the most favorable form of management for spruce stands. The marking plan prepared by J. Kittredge, jr., in 1916, has been followed throughout with a few modifications. Marking was done to reserve single seed trees, seed trees in groups, in strips, and in blocks. The slash has been uniformly piled and burned, most of the burning being done in 1918.

During the summer of 1921 a field examination was made of the area to ascertain the results of cutting. The method used in the field examination consisted of running strips 0.1 chain wide between groups for reproduction and soil surfaces, and chain-wide strips for standing trees and windfalls. Only on those portions of the tract where trees had been reserved singly and in groups had the logging and slash disposal proceeded sufficiently far to permit drawing any conclusions (pl. 6, A and B). In all, 7 strips 5 to 15 chains in length were run. Of these, 4 strips were on areas where the slash was burned in 1920 and 1921 and so could not yield valid data for definite conclusions, though they will furnish the basis for future comparisons. One strip was run 15 chains from a seed group roughly following the contour on a northwestern exposure with a 20 per cent gradient. The survey of this strip is shown in Table VI.

TABLE VI.—*Reproduction of Engelmann spruce by seedlings and by milacres stocked; Ruby Creek area*

[Basis: Strip 15 chains long by 0.1 chain wide, 150 milacres]

Data obtained	Engelmann spruce			Other species			Totals, all surfaces
	Burned	Mineral	Cover	Burned	Mineral	Cover	
Stocking by species and surfaces:							
Number of seedlings on strip	8	46	12	7	28	13	114
Number per acre of actual area	53	303	80	47	187	87	757
Number per acre of each kind of surface [a]	405	2,300	109	356	1,405	118	
Milacres [b] stocked, by species and surfaces:							
Number of milacres on strip	4	19	10	6	25	10	74
Number per acre	26	127	67	40	166	67	[c] 493

[a] Burned surface, 13.2 per cent of actual area; unburned mineral surface, 13.3 per cent; slash and vegetative cover, 73.5 per cent.

[b] Milacre: A quadrat 6.6 feet by 6.6 feet or 0.001 acre.

[c] The total number of milacres stocked with seedlings of each species is 493, but since more than one species may occur on the same milacre, a smaller number of milacres stocked to all species will result. That number is 413.

A.—A spruce seed group reserved after cutting, Ruby Creek area, Pend Oreille National Forest, Idaho

B.—Single seed trees reserved after cutting. Practically all of the single trees left at this point were blown down. The snow mounds in the picture cover upturned tree roots. The picture is taken on a Ruby Creek cut-over area, Pend Oreille National Forest, Idaho

On the basis of this detailed examination of the sample strip, the surface of the whole area that was burned amounted to 13.2 per cent, the unburned 86.8 per cent. Of the unburned soil surface, mineral soil represented 13.3 per cent of the total area, slash cover 15.2 per cent, and vegetative cover 58.3 per cent. The vegetative cover was classified into three categories according to height, and covered the area as follows: Under 5 inches, 31.5 per cent; 5 to 18 inches, 25.4 per cent; over 18 inches, 1.4 per cent.

The vegetative cover was analyzed in this manner to indicate the extent of the soil covering and the rankness of the vegetative growth. Very few species other than those found on the forest floor in the green timber were noted. Considerable stimulation in growth and increase in number of individuals took place following the cutting. *Tiarella* sp. appeared to be the most prolific and comprised the principal portion in the first group.

The duff layer apparently is a very important factor in spruce reproduction. On the area examined nearly 60 per cent of the tract was covered with litter and duff, and measurements showed that this layer varied from 1 to 3 inches in thickness. The duff is found to be most unfavorable to the establishment of spruce seedlings on cut-over tracts. The spruce seed do not hold over or are not stored in the duff. This is contrary to the behavior of the reproduction of western white pine (*Pinus monticola*) and Douglas fir.

A careful analysis of Table VI will indicate the relative favorableness of the various surfaces to the establishment of the spruce. The burned surface yielded 761 seedlings to the acre, of which 405 were spruce; mineral soil bore 3,705 seedlings to the acre, of which 2,300 were spruce; vegetative and slash-covered surfaces yielded only 227 seedlings per acre, 109 of them spruce.

The burned spots were a disappointment. More seedlings were expected to be found on them. This fact agrees, however, with general observations on spots where slash piles were burned.[3] Apparently the soil is sterilized by the concentrated burn. The absence of weeds and grasses has been noticed, and likewise the absence of seedlings. Further study is needed to explain the consistent absence of seedlings on hard-burned spots for the first five years following the burning. Yet the table indicates that burned surfaces are approximately four times as favorable to seedling establishment as duff or litter.

The mineral soil as represented by skidding trails and by the places where the duff layer is completely decomposed is shown by Table VI to be the most favorable surface for the establishment of spruce seedlings. It is nearly six times as favorable as the burned spots and over twenty times more so than the ground covered with litter and vegetation. This is significant and requires recognition in the treatment of spruce stands.

The important indication of Table VI is the unfavorableness of the litter soil cover to the establishment of spruce seedlings. This is corroborated by the results of a study of germination of Engelmann spruce made in 1921 by J. A. Larsen, shown in Table VII.

TABLE VII.—*Germination and survival of Engelmann spruce in different surfaces*

[2,000 seed sowed in each surface, fall 1920]

Germination and survival, 1921	Ashes	Bare soil	Duff
Germination:			
May 15	0	0	0
May 23	429	641	274
June 6	40	27	4
June 20	10	6	[a] 0
July 15	5	15	[a] 0
Total	484	689	278
Percentage of germination	24	34	14
Survival:			
Oct. 10	400	524	2
Percentage of survival	83	76	0.7

[a] On the examination dates of June 20 and July 15, practically all the Engelmann spruce seedlings in the duff were dead. Larsen states that the duff layer was 2 inches deep and attributes the failure of the seedlings to inability of the roots to reach mineral soil before they were overtaken by dry conditions.

Study of the Ruby Creek area indicates that reproduction of Engelmann spruce on cut-over areas is dependent on the seeding of the forest floor at the time of or following cutting. Mineral soil surfaces are found to favor the establishment of spruce seedlings by a ratio of more than 20 to 1 over that of duff surfaces. Reproduction on burned spots is not generally so prolific as that on mineral soil.

[3] 45-acre sample plot, Whitman National Forest, Oregon; Seeley Lake sale area, Missoula National Forest, Montana; Beardmore sale area, Kaniksu National Forest, Idaho.

GENERAL CONCLUSIONS

The essential facts controlling the establishment of Engelmann spruce seedlings as shown by these studies may be briefly stated. The conservation of the surface soil moisture throughout the critical dry period is the essential requirement. Reproduction of spruce from seed deposited in the duff can not be depended on under any conditions. Mineral soil surfaces and lightly burned surfaces decisively favor the quick restocking of Engelmann spruce under favorable moisture conditions; but showers of seed over the cut-over tract must be provided for during several years following cutting.

On the basis of these conclusions the following deductions should govern the method of treatment of spruce stands:

Seed trees must be reserved on cut-over areas, despite possibility of windthrow.

The conservation of soil moisture will require an adaptation of cutting and logging methods to the exposure. On southerly exposures a selective type of cutting is required. On northern exposures an approximate clear-cutting may be practical, but with provision for seed supply.

On protected areas as large a percentage as possible of the duff or litter covering of the soil and the native vegetation must be broken up or burned over. This indicates power logging methods and a more general burning over the area than is secured by burning of piled slash. On areas desiccated by sun and wind logging by horses is required.

In all cases the complete control of slash fires is required. On southerly slopes slash must be piled and burned; on protected areas it may be burned in windrows, with adequate control.

A dense vegetative cover will render the soil as critically dry for spruce reproduction as will an exposed situation.

THE RÔLE OF THE HYDROGEN-ION CONCENTRATION ON THE DEVELOPMENT OF PIGMENT IN FUSARIA [1]

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INTRODUCTION

The present investigations are concerned with the influence of the hydrogen-ion concentration of the substratum on the development of pigment by Fusaria.

The production of pigment has been studied by many investigators and this phenomenon has been used as a character for the taxonomic classification of a number of species. Wollenweber (*14*, *15*),[3] Milburn (*5*) and Bessey (*1*) observed that Fusaria produced different colors if grown on either carbohydrates or proteins, each reaction being accompanied by special colors which changed with the change of reactions of the substratum. The chemical nature of the pigment has probably not been studied, nor the factors which govern its development and intensity. The pigment, which is often observed on Fusaria grown in their natural habitat, is pink, the intensity and hues varying with the substratum.

CULTURAL METHODS

The methods employed for the study of the influence of different hydrogen-ion concentrations on the development of pigment by Fusaria, consisted in growing the organism or organisms in culture media at different initial P_H values and observing the color and intensity of the pigment produced therein. As the studies were concerned with the development of pigment at definite hydrogen-ion concentrations, the use of an apparatus which would permit frequent determinations of the hydrogen-ion concentration of the culture solution became essential. The apparatus, used by the writer (*10*) in certain preceding studies was adopted. It provides for frequent determinations of the hydrogen-ion concentration and for the introduction of various volumes of adjusting reagents, for the maintenance of a fairly constant reaction, under relatively aseptic conditions.

The writer (*11*) as well as others found that Fusaria are capable of changing the reaction of their culture media either by increasing or decreasing the hydrogen-ion concentration, the direction of the changes depending on (*a*) the initial P_H value of the culture solution, (*b*) the chemical nature of the nutrient substance, and (*c*) the age of the culture. On account of this ability of Fusaria to change certain of the initial P_H values of their culture media, the different cultures in the experiment were treated in two different ways—some were adjusted to definite hydrogen-ion concentrations with volumes of 0.2 normal HCl or NaOH, and others were left unadjusted. The purpose of this treatment was to distinguish between the influence of the hydrogen-ion concentration of the culture media and that of the hydrogen-ion concentration of the microorganism, namely, that which is produced by the organism and changes the initial reaction of the culture solution during the assimilation of nutrient substances on the development of pigment.

For the growth of the different organisms and the development of pigment, dextrose solutions in combination with certain inorganic salts were used. They were mixed in the following proportion: Distilled water 1,000 c. c., dextrose 20 gm., $MgSO_4$ 2.12 gm., $Ca(NO_3)_3$ 20.71 gm., KH_2PO_4 1.36 gm., and $Fe(NO_3)_3$ 1 c. c. of a 5 per cent solution. Half-liter portions of this solution were adjusted to definite hydrogen-ion concentrations by the addition of appropriate reagents before being placed in the apparatus mentioned, and sterilized. Solid culture media were also prepared by adding to the above 2 per cent agar-agar.

[1] Received for publication July 26, 1924; issued August, 1925.

[2] Now at the University of Hawaii, Honolulu, Hawaii.

[3] Reference is made by number (italic) to "Literature cited," p. 1019.

DEVELOPMENT OF PIGMENT AT DIFFERENT HYDROGEN-ION CONCENTRATIONS

The development of pigment at different hydrogen-ion concentrations was studied concurrently with the changes produced in the initial P_H value of the different cultures by the organisms.

The determination of the particular color of the pigment in the different cultures were made by using Ridgway's Color Standards (*8*) and those of the hydrogen-ion concentrations of the cultures by using Clark and Lub's (*2*) indicators for relatively clear solutions and Hildebrand's (*4*) hydrogen electrode for heavily colored solutions.

Fusarium cromyophthoron Sid. is treated more extensively in these studies than any of the other species of the same genus. With this organism the development of the pigment and nature of the reactions—that is, decreases or increases in the P_H value—are studied in connection with more nutrient substances than dextrose.

ADJUSTED CULTURES

Changes in the initial hydrogen-ion concentration of the cultures of the first set, the adjusted set of cultures, were neutralized with occasional introductions of 0.2 normal HCl or NaOH for the purpose of maintaining constancy in the initial reaction of the culture. The influence of the reactions of the organism on the initial hydrogen-ion concentration of the culture solutions, possibly affecting the development of pigment, is probably eliminated by the introduction of adjusting reagents in the culture media for the neutralization of the resulting changes in the hydrogen-ion concentration. The changes produced in the initial hydrogen-ion concentration of the culture solution were adjusted regularly (in 4-day intervals) by adjusting reagents, according to the instructions of Sideris .[4]

The results which were obtained in connection with the development of pigment by different species of Fusarium are recorded in Table I.

TABLE I.—*Color, intensity, and diffusibility of pigment produced by different species of Fusarium at different hydrogen-ion concentrations, and volumes of adjusting reagents (c. c. 0.2 normal HCl and NaOH) required to maintain the initial P_H value of the solution constant in dextrose solutions*

Organism	Initial P_H	Adjusting reagent		Color of pigment
		HCl	NaOH	
Fusarium cromyophthoron Sid	3.0	13	0	Pompeian red; madder brown.
Do	4.0	7	0	Van Dyke red.
Do	5.0	0	4	Orange pink (diffusible).
Do	6.0	0	27	Flesh pink.
Do	7.0	0	90	Shrimp pink.
Do	7.5	0	120	Colorless.
F. lutulatum Sher	3.0	10	0	Pomegranate purple.
Do	4.0	0	4	Pale vinaceous.
Do	5.0	0	15	Pale orange yellow.
Do	6.0	0	21	Ivory.
Do	7.0	0	64	Colorless.
Do	7.5	0	112	Do.
F. oxysporum Scht	3.0	10	0	Flesh pink.
Do	4.0	0	10	Pale vinaceous.
Do	5.0	0	15	Venetial pink.
Do	6.0	0	30	Colorless.
Do	7.0	0	45	Do.
Do	7.5	0	90	Do.
F. oxysporum var. *longius* Sher	3.0	17	0	Eugenia red.
Do	4.0	0	5	Vinaceous.
Do	5.0	0	13	Shrimp pink.
Do	6.0	0	25	Colorless.
Do	7.0	0	70	Do.
Do	7.5	0	110	Do.
F. sclerostromaton Sid	3.0	15	0	Acajou red.
Do	4.0	0	3	Alizarine pink.
F. oxysporum var. *resupinatum* Sher.	3.0	10	0	Jasper pink.
Do	4.0	0	7	Pale salmon.
Do	5.0	0	10	Vinaceous lilac.
Do	6.0	0	20	Flesh pink.
Do	7.0	0	65	Colorless.

[4] SIDERIS, C. P. THE INFLUENCE OF THE HYDROGEN-ION CONCENTRATION ON THE DEVELOPMENT OF THE PINK ROOT DISEASE OF ONIONS. (3 PARTS) I. THE EFFECT OF THE HYDROGEN-ION CONCENTRATION ON THE DEVELOPMENT OF FUSARIUM CROMYOPHTHORON SID. [Unpublished thesis, Ph. D., Univ. Calif.

TABLE I.—*Color, intensity, and diffusibility of pigment produced by different species of Fusarium at different hydrogen-ion concentrations, and volumes of adjusting reagents (c. c. 0.2 normal HCl and NaOH) required to maintain the initial P_H value of the solution constant in dextrose solutions*—Continued

Organism	Initial P_H	Adjusting reagent		Color of pigment
		HCl	NaOH	
F. redolens Sher	3.0	12	0	Flesh pink
Do	4.0	0	4	Flesh pink.
Do	5.0	0	10	Capucino orange (diffusible).
Do	6.0	0	30	Colorless.
Do	7.0	0	60	Do.
Do	7.5	0	90	Do.
Fusarium sp. (15)	3.0	11	0	Flesh pink.
Do	4.0	3	0	Do.
Do	5.0	0	5	Ivory.
Do	6.0	0	16	Colorless.
Do	7.0	0	40	Do.
Do	7.5	0	90	Do.
F. malli Taub	3.0	15	0	Pale flesh.
Do	4.0	5	0	Flesh pink.
Do	5.0	0	4	Cinnamon (diffusible).
Do	6.0	0	17	Colorless.
Do	7.0	0	60	Do.
Do	7.5	0	100	Do.
F. angustum Sher	3.0	6	0	Flesh pink.
Do	4.0	0	18	Vinaceous.
Do	5.0	0	26	Shrimp pink.
Do	6.0	0	35	Colorless.
Do	7.0	0	45	Do.
Do	7.5	0	80	Do.
F. loncheceras Sid	3.0	6	0	Salmon pale.
Do	4.0	0	9	Vinaceous, dark.
Do	5.0	0	20	Shrimp pink.
Do	6.0	0	35	Colorless.
Do	7.0	0	70	Do.
Do	7.5	0	130	Do.
F. culmorum (W. Smith) Sacc	3.0	16	0	Begonia rose (diffusible).
Do	4.0	0	3	India red (diffusible).
Do	5.0	0	6	Victoria Lake (diffusible).
Do	6.0	0	23	Colorless.
Do	7.0	0	75	Do.
Do	7.5	0	120	Do.
F. moniliforme Shel	3.0	5	0	Flesh (diffusible).
Do	4.0	0	4	Hydrangia pink (diffusible).
Do	5.0	0	10	La France pink (diffusible).
Do	6.0	0	25	Colorless.
Do	7.0	0	55	Do.
Do	7.5	0	90	Do.
F. radicicola Wr	3.0	21	0	Flesh (diffusible).
Do	4.0	11	0	Scarlet red (diffusible).
Do	5.0	5	0	Peach red (diffusible).
Do	6.0	0	31	Colorless.
Do	7.0	0	75	Do.
Do	7.5	0	125	Do.
F. martii Ap. et Wr	3.0	9	0	Apricot orange (diffusible).
Do	4.0	5	0	Nopal red (diffusible).
Do	5.0	1	0	Maroon dark (diffusible).
Do	6.0	0	36	Colorless.
Do	7.0	0	80	Do.
Do	7.5	0	130	Do.

EXPLANATION OF RESULTS

The results in Table I indicate that the relative hydrogen-ion concentration of culture media of dextrose solutions is possible to initiate or inhibit the development of pigment in Fusaria. Moreover, the color, intensity, and diffusibility of the pigment may be controlled by the relative hydrogen-ion concentration of the surrounding solution. It becomes evident, therefore, that the ability of the different species to form a certain pigment depends, either wholly or in part, on the relative acidity of the culture media. Concentrations of hydrogen-ion higher than P_H of 3.0 or lower than P_H of 7.0 were found to inhibit the development of pigment, practically, in every case. The optimum hydrogen-ion concentrations for the development of pigment lie between P_H of 4.0 and 5.0.

The nature and extent of the changes produced on the initial P_H value of the different cultures by the Fusaria employed, as they are measured by the volume of the adjusting reagent re-

quired to neutralize them, indicate that the organisms reacted amphoteric at hydrogen-ion concentrations between P_H of 3.0 and 5.0. It means, in other words, that the "isometabolic point," (*11*) or the initial P_H value of the culture solution which is not altered by the reactions of the organism during the utilization or assimilation of dextrose, lies between the P_H values of 3.0 and 5.0. The phenomenon of variation in the biochemical behavior of different species, during the assimilation of different nutrient substances, may serve as a character for the differentiation of groups and possibly of species of Fusarium. It is possible, therefore, when one considers (*a*) the behavior of the different organisms at hydrogen-ion concentrations between P_H of 3.0, 4.0, and 5.0, (*b*) the color and intensity of the pigment, and (*c*) the diffusibility of the pigment, to segregate the Fusaria given in Table I into more or less congenial groups. For instance, *F. cromyophthoron*, *F. redolens* and *F. malli*, may be put in one group; *F. radicicola* and *F. martii*, in another; *F. culmorum* and, possibly, *F. moniliforme*, in another, and all the remaining organisms in one more group.

The behavior of *F. cromyophthoron* in onion decoction does not differ from that in dextrose solutions, except in the position of the P_H value of the "isometabolic point" which lies near or at P_H of 5.0, in onion decoction (Table II).

TABLE II.—*Color and intensity of pigment produced by Fusarium cromyophthoron in onion decoction at different hydrogen-ion concentrations and volume of adjusting reagents required to maintain the P_H value constant during the growth of the organism*

Initial P_H value of culture	Adjusting reagents		Color of pigment
	HCl	Na OH	
3.0	20	0	Phlox purple.
4.0	9	0	Van Dyke red.
5.0	0	1	Orange pink.
6.0	0	15	Colorless.
7.0	0	60	Do.
7.5	0	95	Do.

NONADJUSTED CULTURES

These cultures are concerned with the study of the development of pigment by Fusaria in dextrose solutions whose initial hydrogen-ion concentration was not maintained constant by the introduction of volumes of adjusting reagents. The hydrogen-ion concentration of the culture solution which influences the development of pigment in these cultures is not a constant factor but a variable one. This can easily be explained when one considers that the hydrogen-ion concentration of these cultures is not maintained constant by the addition of adjusting reagents, but is subject to the reactions of the metabolic products of the organism. The pigment, therefore, which is developed under these conditions, is subject to the changes which are produced in the hydrogen-ion concentration of the culture solution by the reactions of the organism with the substratum.

The culture media employed for these studies contained 2 per cent agar-agar. They were solid media, prepared in the manner mentioned previously. The organisms were grown in Petri-dish cultures, containing 20 c. c. of the media.

The observations on the development of pigment were made during the first 12 days' growth of the organism on the above culture media, and are recorded in Table III.

TABLE III.—*Color of the pigment produced by different species of Fusarium at different initial hydrogen-ion concentrations of dextrose-agar cultures, during a 10-day growth*

Organism	Initial P_H	Color of pigment
Fusarium cromyophthoron Sid	4.0	Cinnamon pink.
Do	5.0	Cinnamon.
Do	6.0	Cinnamon purple.
Do	7.0	Vinaceous purple.
Do	7.5	Do.
F. redolens Sher	4.0	Cinnamon pink.
Do	5.0	Cinnamon.
Do	6.0	Cinnamon purple.
Do	7.0	Vinaceous purple.
Do	7.5	Do.
F. malli Taub	4.0	Cinnamon pink.
Do	5.0	Cinnamon.
Do	6.0	Cinnamon purple
Do	7.0	Vinaceous purple.
Do	7.5	Do.
F. lutulatum Sher	4.0	Light vinaceous pink.
Do	5.0	Do.
Do	6.0	Shrimp pink.
Do	7.0	Do.
Do	7.5	Shrimp, pale.
F. oxysporum Scht	4.0	Light vinaceous pink.
Do	5.0	Do.
Do	6.0	Shrimp pink.
Do	7.0	Do.
Do	7.5	Shrimp, pale.
F. oxyporum var. *longious* Sher.	4.0	Light vinaceous pink.
Do	5.0	Do.
Do	6.0	Shrimp pink.
Do	7.0	Do.
Do	7.5	Shrimp, pale.

TABLE III.—*Color of the pigment produced by different species of Fusarium at different initial hydrogen-ion concentrations of dextrose-agar cultures, during a 10-day growth*—Continued

Organism	Initial PH	Color of pigment
F. oxysporum var. *resupinatum* Sher.	4.0	Light vinaceous pink.
Do	5.0	Do.
Do	6.0	Shrimp pink.
Do	7.0	Do.
Do	7.5	Shrimp, pale.
F. sclerostromaton Sid.	4.0	Light vinaceous pink.
Do	5.0	Do.
Do	6.0	Shrimp pink.
Do	7.0	Do.
Do	7.5	Shrimp, pale.
Fusarium sp. (15)	4.0	Light vinaceous pink.
Do	5.0	Do.
Do	6.0	Shrimp pink.
Do	7.0	Do.
Do	7.5	Shrimp, pale.
F. angustum Sher	4.0	Flesh pink.
Do	5.0	Do.
Do	6.0	Ochraceous buff.
Do	7.0	Do.
Do	7.5	Pale ochraceous buff.
F. loncheceras Sid	4.0	Flesh pink.
Do	5.0	Pale rose purple.
Do	6.0	Shrimp pink.
Do	7.0	Ochraceous buff.
Do	7.5	Pale ochraceous buff.
F. culmorum (W. Smith) Sacc.	4.0	Hydrangia pink.
Do	5.0	Vinaceous pink.
Do	6.0	Purplish pink.
Do	7.0	Purple.
Do	7.5	Purplish blue.
F. moniliforme (Shel.) Sher.	4.0	Hydrangia pink.
Do	5.0	La France pink.
Do	6.0	Vinaceous pink.
Do	7.0	Vinaceous purple.
Do	7.5	Lilac.
F. radicicola Wr	4.0	Scarlet.
Do	5.0	Peach red.
Do	6.0	Cinnamon purple.
Do	7.0	Greenish purple.
Do	7.5	Greenish blue.
F. martii Ap. et Wr	4.0	Maroon red.
Do	5.0	Nopal red.
Do	6.0	Cinnamon purple.
Do	7.0	Greenish purple.
Do	7.5	Greenish blue.

TABLE IV.—*Color of pigment present in 40-day-old cultures of Fusarium cromyophthoron, grown in dextrose solutions at different initial but nonadjusted hydrogen-ion concentrations, together with determinations of the initial and final P_H value of the different cultures*

Hydrogen-ion concentration of cultures		Color of pigment
Initial P_H	Final P_H	
3.0	4.5	Van Dyke red.
4.0	4.5	Do.
5.0	5.6	Orange pink.
6.0	5.8	Shrimp pink.
7.0	6.2	Pale shrimp pink.
7.5	6.4	Do.

TABLE V.—*Color of pigment present in 40-day-old cultures of Fusarium cromyophthoron, grown in onion decoction at different initial but nonadjusted hydrogen-ion concentrations, together with determinations of the initial and final P_H values of the different cultures*

Hydrogen-ion concentration of cultures		Color of pigment
Initial P_H	Final P_H	
4.0	5.6	Van Dyke, red.
4.7	6.6	Orange pink.
5.4	6.6	Flesh pink.
5.8	6.9	Shrimp pink.
6.2	7.5	Do.
7.3	7.4	Pale shrimp pink.

TABLE VI.—*Color of pigment of sporodochia of Fusarium cromyophthoron, grown on various vegetable tissues*

Vegetable tissues	Color of pigment
Solanum tuberosum L. (tuber).	Pale ochraceous buff to salmon.
Allium cepa L. (bulb)	Pale ochraceous salmon.
Phaseolus vulgaris L. (stems).	Pale ochraceous buff.
Oryza sp. (grains)	Pale ochraceous salmon.
Melilotus alba L. (stems)	Do.

EXPLANATION OF RESULTS

The results given in Tables III, IV, V, and VI indicate that the initial hydrogen-ion concentration of the substratum, if not maintained constant by the addition of adjusting reagents, can not control the initiation or inhibition of pigment, because the reactions produced by the organism during the assimilation of nutrient substances are capable of changing the initial reaction of the culture solution.

Pigment was produced practically in every culture, regardless of the initial hydrogen-ion concentration. It becomes evident, therefore, that the reactions produced by organism or organisms, which, in culture media more acid than the isometabolic point, decreased the hydrogen-ion concentration toward that of the isometabolic point, and in less acid culture media

increased it again toward the same point, were responsible for the formation of the favorable hydrogen-ion concentration for the development of pigment. Another interesting phenomenon, in this connection, is the development of different colors of the same pigment at different hydrogen-ion concentrations. At hydrogen-ion concentrations higher or near that of the isometabolic point the majority of the pigments have a reddish-pink color. At hydrogen-ion concentrations, however, lower than that of the isometabolic point, the same pigments, instead of the reddish-pink color, have a purple, blue, yellow, or green color.

The behavior of *Fusarium cromyophthoron* in dextrose solution and onion decoction at different hydrogen-ion concentrations (Tables IV and V) was practically similar to that observed in solid media; that is, pigment was produced at all the different initial hydrogen-ion concentrations between P_H of 3.0 and 7.5. The slight variation in the color of the pigment of the sporodochia of *F. cromyophthoron* (Table VI) is possibly due to the inherent hydrogen-ion concentration or to the difference in the amounts of carbohydrates and proteins contained in the different tissues. If one takes into account the chemical composition of the tissues in its relation to the color produced in the sporodochia of *F. cromyophthoron*, one finds, according to Wehmer (*12*), that the pale ochraceous salmon color is associated with tissues rich in available carbohydrates and the pale ochraceous buff color with those poor in available carbohydrates but somewhat rich in available proteins.

MOVEMENT OF THE REACTIONS IN SOLID MEDIA

These studies are concerned with the movement of the reactions, particularly those of the hydrogen-ion concentration produced by the different organisms, in solid media.

Dextrose agar media were used for the purpose. They were prepared in the manner mentioned in a preceding paragraph. The culture media were tubed in 20 c. c. portions and sensitized with 10 drops of either one of the indicators used for the determination of different hydrogen-ion concentrations, namely, brom phenol blue, methyl red, and brom thymol blue. Inoculations were made with pure cultures of the different Fusaria in slanted media; and the nature, extent, and movement of the changes in the initial hydrogen-ion concentration were examined daily. The initial hydrogen-ion concentration of the culture media at the time of the inoculation was P_H of 5.0. This method was first adopted by Wolf (*13*) and was used in connection with the changes produced in the hydrogen-ion concentration by certain plant pathogenic bacteria. The changes in the hydrogen-ion concentration were determined by the changes in the color of the particular indicator and are recorded in Table VII.

TABLE VII.—*Changes produced on the color of the different indicators by the reactions of metabolic products of the different species of Fusarium on the hydrogen-ion concentration of the culture media*

Organism	Days of growth	Determinations of the changes in the hydrogen-ion concentration		
		Methyl red	Brom phenol blue	Brom thymol blue
Fusarium cromyophthoron		P_H	P_H	P_H
Sid	4	5.0		
Do	6		3.9	
Do	8		4.5	
Do	10			6.8
Do	12			7.2
F. malli Taub	4	5.0		
Do	6		4.0	
Do	8		4.6	
Do	10			6.0
Do	12			7.4
F. lutulatum Sher	4	5.0		
Do	6		4.2	
Do	8		4.6	
Do	10			6.6
Do	12			7.6
F. oxysporum Scht	4	5.0		
Do	6		3.8	
Do	8		4.2	
Do	10	5.4		
Do	12			5.8
F. oxysporum var. *longious* Sher	4	5.0		
Do	6		4.0	
Do	8		4.4	
Do	10			6.6
Do	12			7.4
F. oxysporum var. *resupinatum* Sher	4	5.0		
Do	6		3.8	
Do	8		4.2	
Do	10			6.8
Do	12			7.6
F. angustum Sher	4	5.0		
Do	6		3.6	
Do	8		4.4	
Do	10			6.6
Do	12			7.6
F. loncheceras Sid	4	5.0		
Do	6		3.6	
Do	8		4.4	
Do	10			6.6
Do	12			7.6

TABLE VII.—*Changes produced on the color of the different indicators by the reactions of metabolic products of the different species of Fusarium on the hydrogen-ion concentration of the culture media*—Continued

Organism	Days of growth	Determinations of the changes in the hydrogen-ion concentration		
		Methyl red	Brom phenol blue	Brom thymol blue
		P_H	P_H	P_H
F. culmorum (W. Smith) Sacc	4	5.0		
Do	6		4.0	
Do	8		4.4	
Do	10			6.0
Do	12			7.0
F. moniliforme (Shel.) Sher	4	5.0		
Do	6		4.6	
Do	8	5.6		
Do	10			6.4
Do	12			7.4
F. radicicola Wr	4	5.0		
Do	6		4.6	
Do	8			6.0
Do	10			6.8
Do	12			7.4
F. martii Ap. et Wr	4	5.0		
Do	6		4.6	
Do	8			5.8
Do	10			6.6
Do	12			7.2

EXPLANATION OF RESULTS

The data in Table VII confirms the results which were obtained on the behavior of Fusarium species in dextrose solutions in all the preceding studies. It is shown that the different organisms increase the hydrogen-ion concentration of the culture media during the utilization of dextrose and decrease it as soon as the supply of this substance is completely removed from the substratum.

The movement of the reactions—that is, the rapidity with which the released hydrogen and hydroxyl ions travel in the different parts of the substratum—is not recorded in Table VII, because the area which manifested the different changes in the hydrogen-ion concentration did not vary in the different cultures. The reactions did not extend much beyond the area which the colony of the different organisms occupied. It may be added that the changes in the color of the indicator, produced by the reactions of the organism in solid culture media contained in test tubes 18 cm. long and 1.5 cm. in diameter, did not extend more than 2 cm. deep from the surface of the colony in the substratum. In cultures, however, older than 20 days the changes in the color of the indicator, and particularly of brom thymol blue, spread throughout the entire culture media. This leads to the conclusion that both the hydrogen and the hydroxyl ions, released in the substratum by the reactions of the organism with the nutrient substances, do not travel fast in solid culture media, because, if they did, the reactions of the hydrogen ions as well as the changes in the color of the indicator would be instantaneous.

Methyl red was far less sensitive than any of the other two indicators. It did not change color after the growth of the organism, this reaction possibly being due to some modification of the methyl red molecule by the metabolic products of the organism.

BEHAVIOR OF DIFFUSIBLE PIGMENTS IN ACIDS AND ALKALIES

The object of these studies was to explain some of the reactions, produced in solid media by certain Fusarium species, which tend to induce the development of pigment of different colors at different hydrogen-ion concentrations.

Diffusible pigments of certain Fusarium species, produced at P_H of 4.0 and 5.0 in dextrose solutions whose initial hydrogen-ion concentration was maintained constant by the addition of adjusting reagents, were treated with 0.1 normal NaOH. The determinations were made with 9 c. c. of the solution containing the diffused pigment against 1 c. c. of the reagent. The changes which were produced in the color of the pigment are recorded in Table VIII.

TABLE VIII.—*Changes produced in the original color of the pigment of certain Fusarium species by the addition of 0.1 normal NaOH*

Organism	Initial P_H	Color of pigment before treatment	Color of pigment after treatment
Fusarium culmorum	5.0	Victoria lake	Dusky auricula purple.
F. radicicola	4.0	Scarlet red	Lilac.
F. martii	5.0	Maroon, dark	Matheus purple.
F. malli	5.0	Cinnamon	Light vinaceous purple.

EXPLANATION OF RESULTS

The results given in Table VIII indicate that the pigment, produced at P_H of 4.0 and 5.0 by certain Fusarium species, if brought in contact with free hydroxyl ions, changes color from reddish pink to blue, green, lilac, or purple. Therefore the blue, green, and purple colors of the pigments of certain Fusarium species, produced at P_H of 7.0 and 7.5, were due to the influence of the hydroxyl ions of the culture media. It becomes evident, from a consideration of the results obtained in the different studies, that the organism or organisms produce the chromogens necessary for the development of pigment at hydrogen-ion concentrations between P_H of 3.5 and 5.5 and that the different colors which are formed from the chromogens later on depend on the hydrogen-ion concentration of the culture media. The mechanism by which these organisms bring about the appropriate reactions in the culture media for the development of chromogens and the formation of pigment of different colors is discussed to a certain extent in the preceding pages and more extensively in a different publication of the writer (*11*).

GENERAL DISCUSSION

The pigments produced by Fusaria belong possibly to the same class of pigments which are produced from chromogens named by Palladin (*6*) "*respiration chromogens.*" According to the same author, these chromogens are glucosides soluble in water and upon the addition of peroxidase and hydrogen peroxide produce red (rarely lilac or violet) color which might change with further oxidation to a dark violet or black. Rupe (*9*) found that an alkaline solution of these chromogens absorbs oxygen very actively, and Combes (*3*) found that the transformation of the chromogen into the pigment is accompanied by increased respiratory activity. Palladin and L'vov (*7*) are of the opinion that these chromogens serve as acceptors of hydrogen. Moreover, they state that they were able to retard the process of alcoholic fermentation by employing the respiration pigment of the white beet to remove the active hydrogen as it was formed.

The pigments produced by Fusaria and those attributed by Palladin to respiration chromogens have certain reactions in common. Both are formed from chromogens during a very active respiration, absorb oxygen very rapidly in alkaline solutions; and are red or pink in acid solutions, and violet, purple, or blue in alkaline solutions.

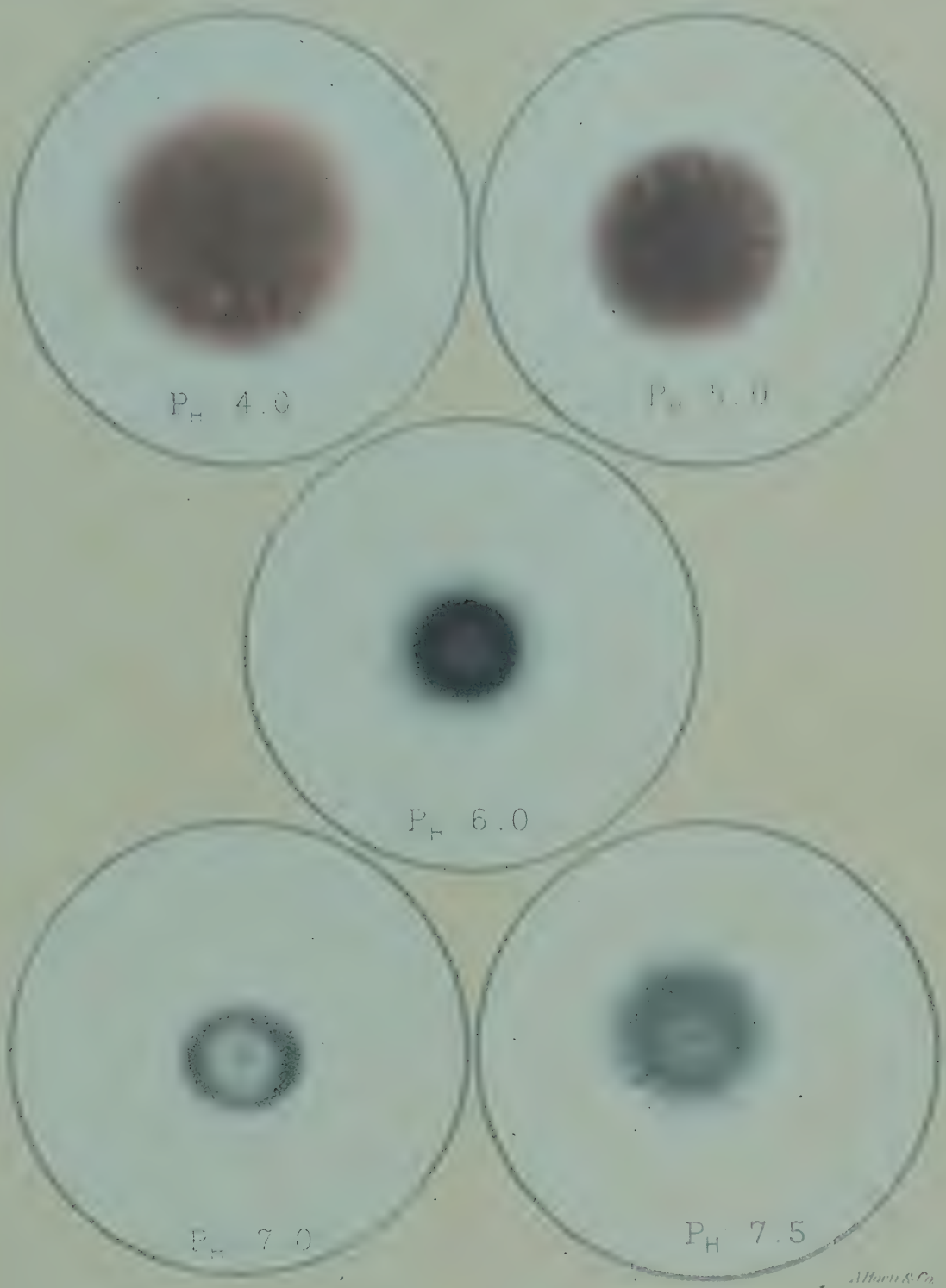
The series of reactions which initiate the development of pigment, during the assimilation of certain nutrient substances by Fusaria, are possibly produced as follows: The organisms, in culture media whose hydrogen-ion concentration is not appropriate for the development of chromogens, may or may not change the initial reaction of the culture media either by increasing or decreasing the hydrogen-ion concentration with their metabolic products. Then, if the hydrogen-ion concentration of the surrounding culture solution is favorable for the development of chromogens, the organisms may produce these substances the color of which in acid solutions may be red, vinaceous, or pink, and in alkaline solutions violet, lilac, purple, blue, or green.

Carbohydrates are essential for the development of pigments. The rôle which they play, in this respect, is not known exactly. They may supply the substances (glucose and organic acids) for the synthesis of glucosides, particularly of the chromogen-glucoside, or the organic acids for the restoration of an hydrogen-ion concentration appropriate for the development of chromogens. The development of chromogens is definitely controlled by the hydrogen-ion concentration of the surrounding solution. In dextrose solutions chromogens may be produced in cultures whose hydrogen-ion concentration is maintained constant by the addition of adjusting reagents at P_H between 3.5 and 5.5, but not above or below these values.

Plate 1 shows the different colors of the pigment produced by *Fusarium culmorum* (W. Smith) Sacc. in solid media at different hydrogen-ion concentrations. The colors from acid to alkali change from red to blue. The chromogen for the blue pigment at P_H 7.5 and the purple at P_H 7.0 was not produced at the above hydrogen-ion concentrations, but at such hydrogen-ion concentrations as were created by the organic acids produced by the organism during the utilization of dextrose. It is possible for the organism, in solid media, to produce a local acidity or alkalinity, that is, a reaction which does not spread throughout the entire culture medium but is confined

EXPLANATORY LEGEND FOR PLATE 1

Fusarium culmorum, grown in solid media at different hydrogen-ion concentrations, namely, P_H 4.0, 5.0, 6.0, 7.0, and 7.5



Washington, D. C.

to the area covered by the colony of the organism, because the movement of the hydrogen ions or hydroxyl ions is very slow and the time required for their neutralization is relatively long. It is easy to see how, with the development of a local reaction, one with a P_H between 4.0 and 5.5 appropriate for the formation of chromogens is possible. The blue color of the pigment produced in alkaline cultures is due to oxidation which is accelerated by the presence of free hydroxyl ions in the surrounding solution.

SUMMARY

The development of pigment by Fusaria is mainly controlled by the hydrogen-ion concentration of the culture media.

Pigment was produced practically by all the different species employed in these studies in dextrose solutions at hydrogen-ion concentrations between P_H 3.5 and 5.5, where the initial P_H value was maintained constant by the addition of adjusting reagents. In cultures, however, whose hydrogen-ion concentration was not maintained constant, pigment was produced at P_H 3.0, 4.0, 5.0, 6.0, 7.0, and 7.5.

The pigment may be of two kinds: Diffusible and nondiffusible, that is, pigment retained within the cell and pigment escaping through the plasmatic membrane and cell wall.

The color which a pigment may take depends on the hydrogen-ion concentration of the surrounding culture solution.

The movement of the hydrogen ions and hydroxyl ions released by the reaction of the metabolic products of Fusaria through solid culture media is very slow.

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EFFECT OF SEEDS UPON HYDROGEN-ION CONCENTRATION EQUILIBRIUM IN SOLUTION [1]

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INTRODUCTION

In an earlier paper [2] it was shown that the hydrogen-ion concentration of alkaline salt solutions in which seeds were immersed had changed markedly after about 15 hours of imbibition. The P_H changes recorded were all in one direction, namely, towards the acid side, and appeared to be fairly definite for each species of seed. The conclusion was drawn that these reaction changes were directly related to ion-absorption by the seeds.

The object of this paper is to show the rate of reaction changes and the existence of a definite equilibrium in solutions which have been in contact with seeds.

EXPERIMENTAL DATA

In view of the fact that a marked difference exists in the absorbing power of seeds of different species (seeds of the leguminous type show higher rates of absorption than seeds of the gramineous species [3]), four kinds of large seeds were selected, as follows: Corn (*Zea mays*), lupine (*Lupinus albus*), beans (*Phaseolus vulgaris*), and soy beans (*Soja maxima*).

The representative salt solutions, mineral and organic acids, covering the range of acids and salt radicles, are given in the tables. In the case of the mineral and organic acids an effort was made to bring the different acids to a dilution giving approximately a reaction of P_H 3.0. Some of the acids could be secured only at a nominal per cent and it was thought best to state the dilution in P_H values instead of in terms of normality. A large number of different salts were tried, but, for the comparison of the radicles, the representative potassium salts are selected and only two chlorides with other bases are entered in the tables.

Fifty seeds of each species were placed in small bottles each containing 100 c. c. of solution; 1.8 c. c. of this solution was pipetted off after definite time intervals, and the hydrogen-ion concentration of the solution was determined by the colorimetric method. The results of these determinations are given in the tables as the averages of at least two trials. In Tables I, II, and III the initial P_H values are compared with the readings at different intervals.

The rate of reaction change is not the same for all salt solutions, although a certain equilibrium is reached in the solutions after the seeds have been immersed sufficiently long (Table I). Corn changed the reaction of KCl to a point of equilibrium in about 15 minutes, while 15 hours were necessary in the case of K_2SO_4. For the mineral and organic acids similar differences were observed.

For mineral and organic acid solutions, considerably more time was necessary to reach the point of equilibrium than for salt solutions (Tables II and III.) On account of the rapid increase in the strength of the acids with every increment of the P_H values, a longer time for the reaction changes could be expected. Nevertheless, all acid solutions which had been in contact with the seeds after a certain period of time reached the same point of equilibrium as the salt solutions.

The P_H values observed in oxalic acid and potassium chloride solutions (corn and beans) plotted against time in minutes are presented in Figure 1. The curves show a fairly rapid rise during the first few time intervals with a flattening out toward the point of equilibrium. Ordinarily the solutions remain at this point indefinitely, but slight changes can be brought about by an increase of temperature. This explains why some of the salt solutions show slightly different P_H

[1] Received for publication Aug. 8, 1924; issued August, 1925. Paper No. 177 of the Journal series, New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

[2] RUDOLFS, W. EFFECT OF SALT SOLUTIONS HAVING DEFINITE OSMOTIC CONCENTRATION VALUES UPON ABSORPTION BY SEEDS. Soil Sci. 11: 277–293, illus. 1921.

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TABLE I.—*Reaction changes in representative salt solutions*

Time	K_2SO_4		K_2HPO_4		KNO_3		$KClO_3$		$CaCl_2$	
	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn
	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H
Initial	5.9	5.9	4.1	4.1	5.4	5.4	6.5	6.4	6.0	6.0
After 30 sec	5.9	5.9	4.1	4.1	5.3	5.3	6.5	6.2	5.9	5.9
After 1 min	5.9	5.7	4.2	4.2	5.5	5.1	6.5	6.1	5.8	5.7
After 2 mins	5.9	5.5	4.2	4.2	5.5	5.2	6.5	5.7	5.8	5.6
After 3 mins	5.9	5.3	4.2	4.1	5.6	5.2	6.5	5.7	5.7	5.6
After 5 mins	6.0	5.4	4.0	4.1	5.7	5.4	6.3	5.4	5.9	5.6
After 10 mins	6.0	5.3	4.1	4.1	5.6	5.1	5.9	5.3	5.9	5.4
After 15 mins	6.0	5.2	4.0	4.0	5.6	4.9	5.9	5.1	5.9	5.4
After 20 mins	6.0	5.0	4.2	4.3	5.7	4.8	5.7	5.1	5.8	5.3
After 30 mins	6.0	5.1	4.4	4.3	5.7	4.8	5.7	5.0	5.8	5.3
After 1 hr	6.0	5.1	4.4	4.3	5.8	4.7	5.7	4.9	5.7	5.3
After 2 hrs	5.9	4.9	4.8	4.5	5.8	4.7		4.9	5.6	5.0
After 15 hrs	5.4	4.2	5.4	4.2	5.9	4.3	5.7	4.2	5.5	4.1
After 48 hrs	5.4	4.2	5.4	4.1	5.8	4.2	5.7	4.0	5.5	4.0

TABLE II.—*Reaction changes in mineral acid solutions*

Time	H_2SO_4		HCl		HNO_3		50 per cent H_3PO_4		H_3BO_3		KCl		$BaCl_2$	
	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn
	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H
Initial	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	5.5	5.5	6.7	6.7	5.9	5.9
After 30 sec	3.1	3.0	3.1	3.0	3.0	3.0	3.1	3.0	5.7	5.4	6.6	6.4	5.9	5.8
After 1 min	3.1	3.0	3.3	3.1	3.1	3.0	3.5	3.0	5.9	5.3	6.5	6.1	5.9	5.6
After 2 min	3.2	3.1	3.5	3.2	3.2	3.1	4.4	3.1	6.0	5.3	6.5	5.2	5.9	5.5
After 3 min	3.2	3.1	3.6	3.2	3.3	3.1	4.5	3.1	6.2	5.3	6.5	4.9	5.9	5.4
After 5 min	3.3	3.1	4.0	3.2	3.5	3.1	4.7	3.1	6.3	5.2	6.3	4.6	5.9	5.3
After 10 min	4.1	3.1	4.6	3.3	3.6	3.2	5.2	3.2	6.4	5.1	6.2	4.1	5.9	5.2
After 15 min	4.6	3.1	4.9	3.4	3.8	3.3	5.7	3.2	6.6	5.1	6.2	4.0	5.9	5.0
After 20 min	4.7	3.1	5.2	3.4	3.9	3.3	5.7	3.5	6.5	4.9	6.1	4.0	5.9	4.6
After 30 min	5.1	3.2	5.5	3.4	4.0	3.3	5.8	3.7	6.5	4.7	5.9	3.9	5.9	4.3
After 1 hr	5.4	3.3	5.7	3.4	4.6	3.3	5.9	4.5	6.4	4.6	5.6	4.0	5.9	4.1
After 2 hrs	5.6	3.4	5.8	3.9	5.4	3.4	5.9	4.4	6.2	4.3	5.7	3.9	5.7	4.1
After 15 hrs	5.8	3.7	5.9	3.9	5.8	3.8	5.9	4.2	6.1	4.2	5.5	3.9	5.5	4.1
After 40 hrs	5.9	3.9	5.8	4.1	5.8	3.9	5.9	4.1	5.9	4.1	5.4	3.9	5.4	4.1

TABLE III.—*Reaction changes in representative organic acids*

Time	Bibasic acid		Hydroxy acids				Fatty acids				Amino acid		Aromatic acid	
	Oxalic		Citric		Lactic		Formic		Acetic		Asparagin		Benzoic	
	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans	Corn	Beans
	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H
Initial	2.9	2.9	2.9	2.9	3.0	3.0	2.9	2.9	2.9	2.9	3.7	3.7	3.2	3.2
After 30 sec	2.9	3.0	2.9	2.9	3.0	3.0	2.9	3.0	2.9	2.9	3.7	3.7	3.3	3.5
After 1 min	3.0	3.1	3.0	3.0	3.0	3.1	3.0	3.1	2.9	2.9	3.7	3.7	3.5	3.7
After 2 min	3.1	3.2	3.0	3.1	3.1	3.1	3.1	3.2	2.8	2.8	3.7	3.8	3.6	3.7
After 3 min	3.1	3.4	3.0	3.1	3.1	3.1	3.1	3.4	2.9	2.8	3.8	3.8	3.6	3.7
After 5 min	3.3	3.9	3.0	3.2	3.1	3.2	3.2	3.6	3.0	3.0	3.8	3.8	3.6	3.9
After 10 min	3.6	4.2	3.0	3.3	3.1	3.6	3.3	4.4	3.0	3.0	3.9	3.8	3.7	4.3
After 15 min	3.7	4.5	3.1	3.7	3.1	3.9	3.5	4.7	3.0	3.0	3.9	3.8	3.7	4.5
After 20 min	3.8	4.8	3.1	3.9	3.1	4.0	3.5	4.9	3.0	3.1	3.9	3.9	3.6	4.6
After 30 min	3.9	5.1	3.1	4.2	3.1	4.2	3.6	5.4	3.1	3.3	4.1	4.0	3.7	4.8
After 1 hr	4.0	5.4	3.2	4.6	3.3	4.5	3.9	5.5	3.1	3.5	4.1	4.4	3.9	5.2
After 2 hrs	4.0	5.6	3.5	4.9	4.0	5.5	4.1	5.8	3.1	3.7	4.2	5.3	3.9	5.5
After 15 hrs	4.1	5.8	3.9	5.7	4.1	5.8	4.1	5.9	3.4	4.2	4.1	5.8	4.0	5.8

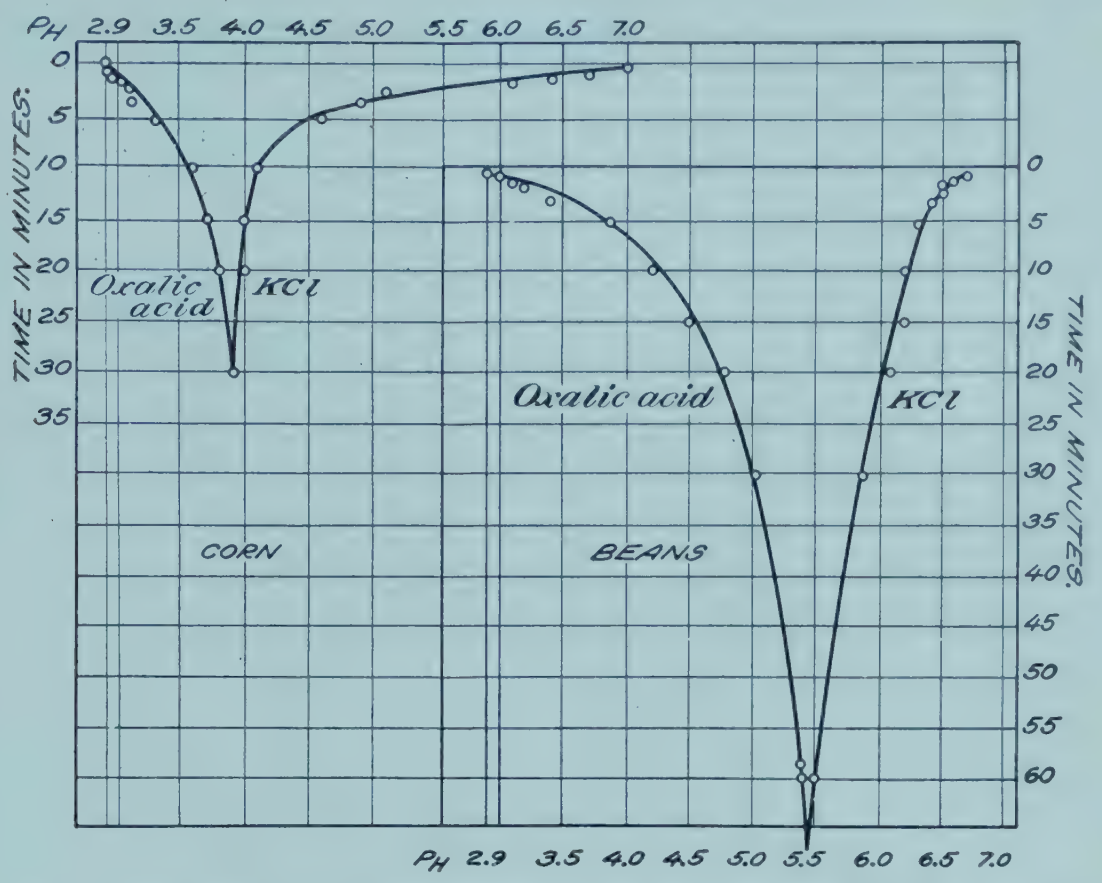

FIG. 1.—Chart showing the P_H values observed in oxalic acid and potassium chloride solutions (corn and beans) plotted against time

values upon standing when the temperature varies. Because variations in temperature cause apparent variations in ion absorption the question might be raised whether or not this is due to the comparative dryness of the seeds at the time of immersion. If the seeds are dry, water intake would presumably be greater and occur at a faster rate than when wet seeds are used. To test this point corn seeds were soaked for a certain period in distilled water before submersion into potassium sulphate. After the immersion a fresh potassium sulphate solution was used again to see when the limit of ion intake was reached. Table IV gives some of the data secured. It will be noticed that the changes produced by previously soaked seeds were very similar to the changes in the case of the air-dry seeds. The same seeds were then quickly taken out of the solution and again placed in a KCl solution of 7 atmospheres osmotic pressure. The observed changes of reaction were not as rapid and extensive as was the case during the first immersion. After 15 minutes the initial P_H value of 5.9 was changed to 5.0 and had risen again after 60 minutes to P_H 5.3. Immersion of these seeds in the same and in fresh solutions for 15 hours caused no further changes. There seems to be no doubt that the equilibrium was reached. Previous soaking in water seemed to have no influence as far as ion intake is concerned.

TABLE IV.—*Corn seeds soaked in distilled water for 30 minutes before submersion into potassium sulphate*

Time	K_2SO_4 (7 atm.)	Fresh solution	Time	K_2SO_4 (7 atm.)	Fresh solution
Minutes	P_H	P_H	*Minutes*	P_H	P_H
Initial	5.9	5.9	After 20	4.1	5.2
After 5	5.1	5.3	After 30	4.3	5.2
After 10	4.9	5.1	After 45	4.2	5.2
After 15	4.3	5.0	After 60	4.2	5.3

After the reaction changes were observed, the first impulse was to hold responsible the rapid changes of life phenomena in the dormant seed when brought in contact with the solutions, or possibly the action of enzymes which are characteristic during the processes of germination. The possibility of changing the salt solutions and acids at such a rate by these agents seemed remote; but, to make sure, seeds were killed by subjection to a temperature of 100° to 102° C. for periods of 48 and 96 hours. To prevent all possibility of enzyme action, beans were subjected to similar temperatures and then placed in formaldehyde. The data secured for seeds dried for 48 hours are given in Table V.

TABLE V.—*Reaction changes in solutions in which "fresh" and dried seed were soaked. The dried seeds were subjected to a temperature of 100° to 102° C. for a period of 48 hours*

Time	NaCl				KCl				Formic aldehyde	
	Lupine		Corn		Lupine		Corn		Beans	
	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried
	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H	P_H
Initial	6.6	6.6	6.6	6.6	6.7	6.7	6.7	6.7	3.6	3.6
After 30 sec	6.6	6.6	6.6	6.6	6.5	6.5	6.4	6.5	3.7	3.6
After 1 min	6.5	6.5	6.3	6.5	6.5	6.3	6.1	6.3	3.8	3.6
After 2 min	6.5	6.5	6.1	6.2	6.5	6.3	5.1	6.3	3.8	3.6
After 3 min	6.5	6.3	5.6	5.8	6.5	6.3	4.9	5.9	3.9	3.6
After 5 min	6.3	6.3	5.4	5.7	6.3	6.3	4.5	5.5	4.0	3.7
After 10 min	6.3	6.3	4.9	4.9	6.3	6.3	4.1	4.9	4.3	3.9
After 15 min	6.3	6.3	4.6	4.4	6.2	6.3	4.0	4.7	4.5	4.1
After 20 min	6.3	6.3	4.4	4.3	6.3	6.1	4.0	4.2	4.7	4.4
After 30 min	5.9	6.2	4.1	4.1	5.9	6.1	3.9	4.1	4.9	4.8
After 1 hr	5.5	6.3	4.1	4.1	5.5	6.1	3.9	3.9	5.0	4.9
After 2 hrs	4.9	4.9			4.9	5.2			5.4	5.3
After 18 hrs	4.7	4.6			4.7	4.6				

A study of this table shows that the reaction changes were possibly somewhat slower in the dried seeds but that in general the reaction velocity did not undergo great changes. It might be that because of the drying of the seeds, slight chemical changes occured in the seed, so that the mechanical intake of the ions was retarded; or it might be that, on account of the drying, some of the retained moisture in the seeds was driven out and the moisture content had to be replaced to its original amount before the seeds were able to absorb the ions from the salt solutions and acids. It is interesting to note, however, that in all cases the final equilibrium was established regardless of the previous drying. Even in the case of dried seeds soaked in a formaldehyde solution the hydrogen-ion concentration was changed from P_H 3.6 to P_H 5.3 after 2 hours. None of the dried seeds germinated, while from 61 to 84 per cent of the "fresh" seeds germinated after being subjected to immersion in salt solutions for 2 hours.

It is known that when dry seeds are placed in moist soil or salt solutions they absorb moisture with great power. This absorption is not a simple phenomenon but implies forces like imbibition, capillarity, surface tension, osmotic pressure from internal salts, and possibly other forces. The amount of absorption depends on the salt concentration in the soil or solution. In previous papers [4] it has been shown that there is a difference in the absorbing powers of different species of seeds, that different salt solutions are differently affected, and also that there is variation in the amounts of salt solutions (in the form of ions) taken up by the different seeds.

Loeb [5] has suggested that in amphotheric membranes like those in the protoplasm of root hairs, and of vacuolate cells generally, the opposite sides of the membrane may be oppositely charged. Many different kinds of membranes are semipermeable and the property of all in common in that they are colloidal gels. Water can penetrate both phases of the colloidal gel, but salt molecules attempting to penetrate the membrane would be prevented by physical phenomena. From the data here presented, it seems clear that the ions of the solutions are rapidly absorbed by the seeds, but the material which makes up the seeds, and especially the seed coats, can not directly be compared with the colloidal gel or the semipermeable membrane of the cells of root hairs.

A study was therefore conducted to determine what part of the seed plays the most important rôle in ion ab-

[4] RUDOLFS, W. EFFECT OF SALT SOLUTIONS HAVING DEFINITE OSMOTIC CONCENTRATION VALUES UPON ABSORPTION BY SEEDS. Soil Sci. 11: 277–293, illus. 1921.
——— EFFECT OF SEEDS UPON HYDROGEN-ION CONCENTRATION OF SOLUTIONS. Bot. Gaz. 74: 215–220. 1922.

[5] LOEB, J. THE REVERSAL OF THE SIGN OF THE CHARGE OF MEMBRANES BY HYDROGEN IONS. Jour. Gen. Physiol. 2: 577–594, illus. 1920.

sorption. The seed coats of soy beans were carefully removed from the cotyledons and both cotyledons and coats placed in different acids and salt solutions; similar material was placed in distilled water. The water was not redistilled and was, as usual, slightly acid. The figures secured for beans soaked in hydrochloric acid are presented in Table VI.

TABLE VI.—*Reaction changes in 0.001 normal HCl and distilled water caused by dicotyledons of soy beans*

Time	HCl		Water	
	Coats	Dicotyledons	Coats	Dicotyledons
	P_H	P_H	P_H	P_H
Initial	3.6	3.6	6.6	6.6
After 30 sec	4.7	5.0	6.6	6.6
After 1 min	4.8	5.4	6.6	6.6
After 2 min	5.2	5.7	6.6	6.6
After 3 min	5.3	5.9	6.6	6.7
After 5 min	5.5	6.0		
After 10 min	5.6	6.0	6.7	6.6
After 15 min	5.9	6.1		
After 20 min	6.0	6.1	6.6	6.6
After 30 min	6.1	6.1	6.6	6.6
After 60 min	6.1	6.1	6.6	6.6

It can be seen at once that the cotyledons of the soy beans were more powerful in absorbing ions from this acid than were the coats. No reaction changes seemed to occur in distilled water. The dicotyledons of soy beans contain saturated acids, great amounts of oil and masses of protein. Since proteins are amphotheric, it seems justifiable to assume that the proteins are mainly active in ion absorption. It is well known that carbohydrates do not behave in a way similar to proteins. However, the influence of corn seed coats, the carbohydrates of the inner cells of the seeds, and pure starch upon the reaction changes of magnesium sulphate solutions as compared with redistilled water was determined. Table VII gives the condensed data secured in one series of trials.

The reaction changes brought about by the seed coats in the $MgSO_4$ solutions were similar to the reaction changes caused by the whole seeds, while the reaction changes caused by the endosperm of the corn seeds (carbohydrates mainly) were negligible. As could be expected, no changes occurred in the salt solutions or water with pure starch. The protein content of the corn seed coat seemed, therefore, responsible for the changes of hydrogen-ion concentrations.

It may perhaps be said that the characteristic external acidity which each species of seeds tends to preserve seems to be determined by the chemical properties of its chief constituent protein. The fact that a given species of seeds causes (and maintains in weak solutions) a certain equilibrium of hydrogen-ion concentration might possibly throw some light upon the question why certain plants are better able to withstand an acid or alkaline soil than others. If for instance a certain species of seeds maintains a characteristic P_H point 4.0, the seedling possibly would be able to survive in a more acid soil than a species of seeds with a characteristic P_H point of 5.8. However, a certain equilibrium caused by the seeds in the surrounding nutrient or soil solution does not necessarily mean that the growing plant causes a similar reaction, because the watery protein materials in the growing plant cell might have different characteristic P_H points. Moreover, the growing plant seems to be able to adjust or regulate internal changes readily. This is shown by Bauer and Haas,[6] who

TABLE VII.—*Influence of corn seed coats and starch upon the reaction of changes of magnesium sulphate as compared with distilled water*

Time	$MgSO_4$		Water		Pure starch	
	Coats	Endosperm	Coats	Endosperm	$MgSO_4$	Water
	P_H	P_H	P_H	P_H	P_H	P_H
Initial	6.7	6.7	7.0	7.0	6.7	7.0
After 15 min	4.6	6.7	6.9	6.9	6.7	7.0
After 30 min	4.2	6.6	6.9	6.9	6.7	7.0
After 60 min	4.2	6.5	6.8	6.9	6.7	7.0

[6] BAUER, F. C., and HAAS, A. R. C. THE EFFECT OF LIME, LEACHING, FORM OF PHOSPHATE AND NITROGEN SALT ON PLANT AND SOIL ACIDITY, AND THE RELATION OF THESE TO THE FEEDING POWER OF THE PLANT. Soil Sci. 13: 461–477, illus. 1922.

state: "In the case of the soy bean roots, while the hydrogen-ion concentration usually showed a direct relation to the acidity of the soil, the total acidity (of the plant) usually varied in the opposite direction. The data of these experiments strikingly show the power possessed by plants to regulate internal acidity. Marked differences in the acidity of the soil caused only small differences in the acidity of the plant juices." It might be that the acidity or alkalinity of soils as such is not always the limiting factor in soil productivity. It is recognized that the measure of soil acidity alone may be useful in determining the amount of lime necessary to adjust a soil for a crop, but the establishment of the characteristic P_H point for the seeds as revealed in these tests together with the determination of the hydrogen-ion concentration of the soil solution might serve as a better indicator in the use of particular crops in particular soils.

SUMMARY

When seeds were immersed in representative salt solutions, mineral and organic acids, and the changes in hydrogen-ion concentration and reaction changes were recorded, it was found that different seeds are able to change the hydrogen-ion concentration of the solutions to definite points, and that certain equilibrium is reached in all solutions after the seeds have been immersed sufficiently long. The changes of the solutions in which previously soaked seeds were immersed are very similar to the changes in solutions caused by air-dry seeds.

The reaction caused by dried seeds (dried at 100° to 102° C. for 48 and 96 hours) are similar to the reaction changes caused by fresh seeds, although the rate of reaction is slightly less. The cotyledons of soy beans were more powerful to absorb ions from the solutions than were the seed coats; and the reaction changes caused by seed coats of corn were similar to the changes brought about by the whole seeds, while changes caused by the endosperm of the seeds (carbohydrates mainly) were negligible. The chemical properties of the chief protein constituent of the seeds seem responsible for the changes in hydrogen-ion concentrations of the solutions.

FUSARIUM RESISTANT CABBAGE: PROGRESS WITH SECOND EARLY VARIETIES[1]

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INTRODUCTION

About 25 years ago E. F. Smith (*7*),[2] while studying the bacterial black-rot disease of cabbage, recorded another disease which he correctly diagnosed as caused by a vascular Fusarium. In this connection he pointed out the serious danger from this and similar soil-borne fusarial diseases. Apparently this disease was not then widespread in its occurrence and little further attention was given to it until about 10 years later. Then Harter (*2*) noted its serious development in the southeastern States and Manns (*6*) reported it as becoming highly destructive in the intensive cabbage-growing districts of Ohio. It was about this time that the senior writer learned that the cabbage growers along the shores of Lake Michigan from Milwaukee southward into the environs of Chicago were being forced out of the cabbage industry because of the rapidly increasing inroads of this "yellows" disease and sent a culture of the fungus to Wollenweber (*10*), who described and named the species *Fusarium conglutinans.*

Since then evidence has rapidly accumulated to show that this parasite has become established across the continent to California on the west and to the Gulf States on the south. Its occurrence from Iowa and southern Minnesota across the northern States to southern New York has been a matter of frequent report for several years. It was found by Walker to be destructive near Mobile, Ala., in 1918, and in 1923 H. D. Barker, in correspondence, reported it as serious in central Mississippi. In 1922 Monteith observed it on kale at Santa Rosa, Calif.; in 1923 Walker observed it in the Greeley district of Colorado, causing a 25 per cent loss in one field; and Stokdyk (*8*) has reported it from Kansas.

Moreover, in accord with Smith's warning, wherever introduced it has soon become the most seriously destructive of all cabbage diseases in those regions where climatic conditions favor its development. It has, however, been further shown (*1, 8, 9*) that this disease, like most of those caused by vascular Fusaria, is distinctly limited by soil-temperature relations. It is a high-temperature parasite, and since the invasion occurs only through the root system when the soil temperature remains below about 17° C. its attacks are inhibited, whereas above this temperature the severity of the disease increases rapidly with rise in temperature for about 10°. These temperature influences are especially potent in the northern States during the earlier growth of the plant when it is relatively shallow-rooted, the periods of most serious susceptibility being (*a*) the seedling stage and (*b*) the time immediately following transplantation.

Previous publications (*3, 4*) have demonstrated the practicability of controlling this disease through the selection of disease-resisting strains and have recorded the earlier successes with certain of these as used locally in the vicinity of Racine, Wis., and Chicago, Ill. More recent developments have confirmed the earlier judgments as to the futility of turning to any other method of control of the cabbage Fusarium disease. At the same time they have indicated the pressing and widespread national importance of developing and of securing the adequate maintenance of disease-resisting strains. At first it seemed that perhaps two or three such strains of different types would meet the outstanding needs.

[1] Received for publication July 29, 1924; issued August, 1925. The writers wish to acknowledge the assistance of W. B. Tisdale, now of the Florida experiment station, who was associated with this work from 1919 to 1922, and of E. C. Tims, who assisted in the conducting of field trials in 1922 and 1923 and has conducted other investigations of the problem to be reported in an independent publication entitled, "Studies on the Fusarium Disease of Cabbage" (Ph. D. thesis, Univ. of Wis., 1924). Appreciation is also expressed to the National Kraut Packers' Association for generous financial support, and to Martin Meeter, chairman of the seed committee of that association, for his hearty cooperation.

[2] Reference is made by number (italic) to "Literature cited," p. 1034.

This was upon the assumption that the disease was localized. Now that it is recognized as essentially national in its distribution, urgent appeals are coming from the several commercial cabbage-growing interests for a correspondingly diversified range of types to meet their various local or regional needs.

This publication is presented as a report of progress since the date of the last detailed article (*4*) in the writers' efforts to meet these needs. In order to link the present publication with the former, it will be necessary to state briefly the place now held by the previously distributed resistant strains and to review their behavior under commercial conditions.

PRESENT STATUS OF RESISTANT STRAINS PREVIOUSLY DISTRIBUTED

At the time of the last detailed publication (*4*) there were two strains of the Ball Head or Hollander type of winter or storage cabbage. Of these the first was distributed as Wisconsin Hollander, but upon the subsequent development of an earlier selection was renamed Late Wisconsin Hollander in order to distinguish it from the other, which was named the Early Wisconsin Hollander. This earlier, shorter-stemmed strain was selected from the parent Wisconsin Hollander strain by W. J. Hansche, an expert cabbage grower and dealer of Racine.

In addition to these two resistant strains of the winter or storage cabbage, two other resistant strains of cabbage of the mid-season or domestic type had been obtained. These were distributed under the names Wisconsin All Seasons and Wisconsin Brunswick. Efforts with these four strains during the past five years have been directed primarily toward the guidance of those interested or engaged in the commercial growing and distribution of seed. Trials on sick soil have been made annually, using either seed of these resistant strains from stocks grown by Wisconsin farmers or similar seed from stocks of the commercial seed trade which had been secured by increasing the Wisconsin resistant stock through growing it for one generation in one or another of the commercial seed-growing sections.

During this period no noticeable deviation or loss in resistance in any of these strains has been found where the seed was grown successively from heads selected from sick soil. Nor has any appreciable reversion been noted where such seed is increased properly for a single generation in a yellows-free commercial seed region. The effect of repeated multiplication in a disease-free area is not known, but it is to be expected that it would in time lead to considerable loss in resistance through the reproduction of a small but probably increasing percentage of susceptible plants (*5*).

Through the development of the four above-named varieties, the leading demands for resistant strains of late storage and medium late domestic cabbage have been met. Through succeeding years reselection of these varieties should continue by those interested, and from them special strains adapted to various localities should result. As noted above, this has already occurred in the selection of the Early Wisconsin Hollander at Racine by Hansche; and independently of this work Bugner, of Chicago, has selected another resistant strain of Hollander which is now offered in trade channels. In the same way further selection and possible improvement by others should naturally proceed with these later varieties. The writers' attention since 1919 has been directed toward the development of resistant strains from earlier varieties of cabbage to meet the needs of kraut manufacturers and cabbage growers in general.

SELECTIONS FROM SECOND-EARLY VARIETIES

Using the methods already described, the selection was started in 1919 from two standard second-early types—one a flat-head type, the other a round-head type. For the first the standard variety All Head Early was used as the original stock; and for the second, two very similar varieties, Glory of Enkhuizen and Copenhagen Market. The All Head Early is some two weeks earlier than All Seasons, is not as rank and leafy a plant as the latter, and produces a somewhat flatter head. The Glory of Enkhuizen requires about the same length of season as All Head Early, has fewer outer leaves than All Seasons, and produces a prominent spherical head. The original Copenhagen Market was of the same general type as Glory of Enkhuizen, but somewhat earlier. There are now so many strains of the Copenhagen Market, however, that the two names are used more or less interchangeably. At least the strains of both varieties with which the writers have worked are so similar that it will probably not be advisable to carry them farther as distinct varieties. None of these selec-

tions have the extreme earliness of certain Copenhagen strains, but are in line with later strains of this variety and with the generally accepted Glory of Enkhuizen type.

The writers have had to contend with greater practical difficulties inherent in methods of handling the early varieties as compared with the late-season varieties. These results from the fact that in the first place, in order to secure evidence as to both relative disease resistance and horticultural type, the plants must be brought to maturity in late summer rather than autumn; and in the second place, plants matured so early, especially those of the earlier soft types, do not keep well in winter storage. Many of the selections in the early years of the work were lost during winter storage, and this loss was naturally most severe with the earlier maturing individuals.

During the last three years this difficulty has been overcome to a considerable degree in two ways. In the first place, the resistant plants, after having passed the severe yellows period in midsummer and having sufficiently approached maturity to permit of critical selection for earliness and type, are "lifted" from time to time sufficiently to break part of the root system and thus delay final maturation. They can thus be allowed to remain in the field, under Wisconsin conditions, until freezing weather, when winter cabbage is naturally placed in storage. By thus shortening the storage period, greater success is insured. In the second place, a small number of the best heads, after six to eight weeks of dormancy in the field or in storage, are planted in the greenhouse, where with proper handling a seed crop may be matured in May and June. The latter method has the especial advantage of making it possible to try out the new strains immediately. It also has the advantage of facilitating hand pollination both for selfing and crossing.

SELECTIONS FROM ALL HEAD EARLY

Two series of selections from All Head Early have been carried through the second generation. The original stock in each case was from a lot of seed grown by Linnaeus Allen at Cutchogue, Long Island. The original selections of the first series were made from a thoroughly sick field at Union Grove, Wis., in 1919. The second series was selected from rows grown in trial plots at Racine, Wis., in 1920. The behavior of the resulting strains on sick soil at Racine is summarized in Table I. In each season's trials there was included for comparative purposes a susceptible commercial strain of the variety and the Wisconsin All Seasons, which is one of the most highly resistant types. The trials extended over the years 1921 to 1923. Some variation between the seasons in the severity of yellows is evident. Judging from the behavior of the commercial strain and of Wisconsin All Seasons, the year 1921 afforded the severest test, while that of 1923 was perhaps the least severe. It is well to keep this point in mind in comparing the behavior of the different series reported.

Heavy mortality of seed plants reduced the trials in Series I to a single strain in each generation. It will be seen from the data in Table I that considerable resistance was already shown in the first generation (XL–20–1 (pl. 1, A), though it was not equal to Wisconsin All Seasons. Of the selections from this lot, only one seed plant survived, and, having grown in isolation, it was probably self-pollinated. In the trial of this strain (XL–22–1) no yellows whatever was found in a large population, although a trace of the disease might have resulted in a warmer season (pl. 1, B). The plants in this trial were unusually uniform, but unfortunately they deviated so widely from the characteristic All Head Early type as to be of little value. They were of a yellowish green rather than the characteristic dark green color; the heads matured much later than All Head Early, and had a decided tendency to become peaked as they matured. This example illustrates the great danger of rapid digression from the desired type in the selection of cabbage which, being normally cross-pollinated, is probably homozygous in few if any of its characters.

In the second series less rapid advance in the acquisition of resistance was made. All strains showed a high percentage of yellows in the Broesch plot and somewhat less in the Drummond plot. The difference in disease occurrence between the two plots is probably due to the fact that two types of soil are represented, which may vary somewhat as to temperature as well as other factors. From the five first-generation strains tried, one (XL–21–5) was picked for further selection, since it developed the best type of plant, while it was nearly equal to XL–21–4 and superior to the other strains in resistance. Of the 26 heads selected from XL–21–5, four plants produced seed in the greenhouse, and these

TABLE I.—*Behavior of selections from All Head Early cabbage when grown on yellows-sick soil at Racine, Wis.*

Series No.	Generation	Strain No.[a]	Year mother head selected	Year seed grown	Year strain tested	Behavior on affected soil: Broesch plot: Total plant	Broesch plot: Per cent yellows	Broesch plot: Per cent headed	Drummond plot: Total plants	Drummond plot: Per cent yellows	Drummond plot: Per cent headed	Remarks
I	First	XL–20–1	1919	1920	1921				69	33	68	5 heads selected.
		Wisc. All Seasons							135	18	84	
		Commercial All Head Early.							135	99	0	
	Second	XL–22–1	1921	1922	1923	507	0	92	934			Decidedly off type.
		Wisc. All Seasons				258	1	83				
		Commercial All Head Early.				213	90	15	138	72	12	
II	First	XL–21–1	1920	1921	1922	157	75	22	18	44	55	Discarded.
		XL–21–2				231	69	42	215	58	70	Do.
		XL–21–3				245	66	47	121	48	82	Do.
		XL–21–4				263	64	22	121	25	94	Do.
		XL–21–5				265	71	47	120	34	94	26 heads selected.
		Wisc. All Seasons				364	8	96	119	3	99	
		Commercial All Head Early.				105	77	12	142	75	42	
	Second	XL–23–1	1922	[b]1923	1923	113	41	44				Good; discarded.
		XL–23–2				91	32	62				Type good; preserved.
		XL–23–3				63	22	66				
		XL–23–4				49	20	73				Very good type; preserved.
		Wisc. All Seasons				258	1	83				
		Commercial All Head Early.				213	90	15				

[a] The method used in numbering the cabbage strains is as follows: A Roman numeral has been used consistently throughout the trials to designate the original commercial variety from which the selection was made, XL being this numeral for All Head Early. The pair of Arabic figures next appearing, i. e., between the dashes (–21–, etc.), indicates the year (1921, etc.) when the seed was grown, and the last figures is the number of the particular strain in question.

[b] Seed was greenhouse grown.

second generation strains were tried out in 1923. A considerable improvement in resistance was shown by these strains, and contrary to the results in Series I, the type was quite satisfactory (pl. 1, B). Strain XL–23–1 was discarded because of its poor resistance, but the remaining three were preserved for further selection.

SELECTIONS FROM THE GLORY–COPENHAGEN TYPE

Four separate series of selections have been made from the Glory-Copenhagen type. The results of the work to date are summarized in Table II. In each case the original selections were made from plantings of the standard variety on very sick soil, either in commercial fields or from experimental trial plots. Series I was selected from a commercial field at Union Grove, Wis.; Series II and III were selected from commercial rows in the trial plots at Racine, Wis.; and Series IV was selected from commercial rows in a demonstration plot conducted by F. D. Fromme at Marion, Va.

Trials on sick soil with seed from the first-generation selections have been made in each case. In every trial a commercial strain of Glory of Enkhuizen and a strain of Wisconsin All Seasons were included. These trials extended over the years 1921 to 1923, and the variation between these seasons in the severity of yellows is again to be noted. In no case have any of the first generation strains equaled the Wisconsin All Seasons in resistance. On the other hand, it will be seen by comparison that a marked advance has been made over the commercial strain in the first selection. In fact, the strains in Series III and IV are at a point where as such they are of distinct commercial value.

TRIALS OF SELECTIONS FROM ALL HEAD EARLY ON YELLOWS-SICK SOIL

A. View of Drummond plot, Racine, Wis., in 1921. The row in the center was planted with commercial All Head Early; note that the yellows disease has killed nearly every plant. The next row to the left is a *first generation* selection from All Head Early, XL–20–1. Note the nearly complete stand as compared to the control. The remaining rows to the left are the resistant Late Wisconsin Hollander. The first two rows to the right of the control are strains of the resistant Maryland Flat Dutch

B. View of the Broesch plot, Racine, Wis., in 1923. The row in the center is commercial All Head Early; note that here, as in field A, this is nearly destroyed by disease. The first row at the left of the control is the progeny of a single plant (XL–22–1) selected from the All Head Early selection, XL–20–1, tried in 1921 and shown above in A. The second generation selection proved to be highly resistant, but is of undesirable type, being too late in maturity, too peaked as to head shape, and too yellowish green as to color. The first row on the right of the control contains second-generation selections from All Head Early (XL–23–1 to 5), belonging to a line distinct from those noted above. These strains are also highly resistant and approximate more closely the desired type. These give promise of commercial value

TRIALS OF SELECTIONS FROM GLORY OF ENKHUIZEN-COPENHAGEN MARKET TYPE UPON YELLOWS-SICK SOIL

A. View of Drummond plot, Racine, Wis., in 1923. The row at right center is commercial Glory of Enkhuizen; note that the yellows disease has killed most of the plants. The four rows at the left are first generation selections (XXX 22-1 to 4) which are highly resistant

B. View of the Broesch plot, Racine, Wis., in 1923. The control row in the center is commercial Glory of Enkhuizen, which suffered badly from disease. The several rows at the right are of the same first generation selections shown in A (Series IV, XXX-22-1 to 6). At the left are first generation selections of Series III, XXXV-21-1 to 6. Note that in each case the selected strains show high degrees of resistance as compared with the commercial control of the same variety

TABLE II.—*Behavior of selections from Glory of Enkhuizen–Copenhagen Market strains of cabbage when grown on yellows-sick soil at Racine, Wis.*

Series No.	Generation	Strain No.	Year mother head selected	Year seed grown	Year strain tested	Behavior on affected soil: Broesch plot: Total plants	Broesch plot: Per cent yellows	Broesch plot: Per cent headed	Drummond plot: Total plants	Drummond plot: Per cent yellows	Drummond plot: Per cent headed	Remarks
I	----------	Commercial Glory	------	------	1921	-----	-----	-----	260	98	2	
	----------	Wisc. All Seasons	------	------					135	18	84	
	First	XXXV–20–1 [a]	1919	1920					288	43	56	Poor; discarded.
		XXXV–20–4							184	42	64	Fairly good type; preserved.
II	----------	Commercial Glory	------	------	1922	123	92	27	123	82	37	
	----------	Wisc. All Seasons	------	------		364	8	96	-----	-----	-----	
	First	XXXV–21–1	1920	1921		128	30	70	11	27	95	Fairly good type; preserved.
III	First	XXXV–22–1	1921	1922	1923	246	16	72	737	17	67	Poor type; discarded.
		XXXV–22–2				254	18	72	925	19	68	
		XXXV–22–3				255	20	75	555	17	76	Good type; preserved for further selection.
		XXXV–22–4				254	14	80	652	16	73	
		XXXV–22–5				257	21	----	647	12	80	
		XXXV–22–6				513	15	73	919	15	80	
	----------	Wisc. All Seasons	------	------		258	1	83	287	1	85	
	----------	Commercial Glory	------	------		251	73	12	401	68	18	
IV	First	XXX–22–1	1921	1922	1923	250	14	79	726	16	75	Good type; preserved for further selection.
		XXX–22–2				249	16	79	549	15	82	
		XXX–22–3				253	10	85	241	14	79	
		XXX–22–4				248	18	78	462	11	83	
		XXX–22–5				254	14	80	342	11	75	Poor type; discarded.
		XXX–22–6				133	14	80	123	10	28	
	----------	Wisc. All Seasons	------	------		258	1	83	287	1	85	
	----------	Commercial Glory	------	------		251	73	12	401	68	18	

[a] The method used in numbering the cabbage strains is as follows: A Roman numeral was used to designate the original commercial variety from which the selection was made—XXX for Copenhagen Market and XXXV for Glory of Enkhuizen. The pair of Arabic figures next appearing—i. e., between the dashes (–20–, etc.)—indicate the year (1920, etc.) when the seed was grown, and the last figure is the number of the particular strain in question.

As to type, variation between strains is evident throughout. In Series I, strain XXXV–20–1, though showing a fair degree of resistance, was of inferior type and was therefore discarded. Although the other strain in the series, XXXV–20–4, was somewhat better, and further selection from it has been made, it is likely that it will eventually be discarded in favor of more satisfactory strains. Selections from the single strain of Series II are being continued for further study. The last two series, III and IV, offer the greatest promise (pl. 2). Of the six head strains in each, two from each series have been discarded because of deviation from the desired type. From the remaining strains, by rigid selection, several hundred typical heads have been preserved for further trials and for stock seed production.

SUMMARY

The progress with the selection of yellows-resistant second-early varieties of cabbage as here reported thus far confirms the writers' earlier belief that from any of the standard varieties of cabbage desirable resistant types may be obtained. While the work with the All Head Early and the Glory-Copenhagen types has not yet reached a finished state, the writers feel that they have advanced to the stage where the selections are approaching commercial value. They shall continue to make such improvements as are possible both as to type and as to resistance, and it is hoped that within a reasonable period these new strains will find their place in the regular commercial seed trade channels.

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THE COTTONY LEAK OF CUCUMBERS CAUSED BY PYTHIUM APHANIDERMATUM[1]

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INTRODUCTION

While several species of Pythium, notably *Pythium debaryanum* Hesse, have been found destructive to a wide variety of phanerogams in the seedling stage, and inimical to the best development of some of these hosts in later stages, the association of the genus with decay of commercial vegetable products, representing parts of plants approaching maturity, has not been frequently recorded. Perhaps the most generally known instance is represented by the "leak" of potato (*Solanum tuberosum* L.) tubers, apparently encountered by De Bary (*2*)[2] in Germany more than four decades ago, and more recently made the subject of special study in the United States by Hawkins (*8*). A soft rot of sweet pepper (*Capsicum annum* L., var. *grossum*) has been recorded by Lehman (*9*) from North Carolina as being due similarly to *Pythium debaryanum*, the decay always beginning at the blossom end, and affecting fruits not more than 6 or 8 inches from the ground. The same fungus is mentioned in the list of fungi thriving on fruit in Belgium by Él. and Ém. Marchal (*11*), who observed it on a pear (*Pyrus communis* L.) lying on damp ground.

MATERIAL EXAMINED

This paper deals with a disease of cucumber (*Cucumis sativus* L.) fruit which the writer first observed in specimens submitted to him June 8, 1922, by the food products inspector of the Bureau of Agricultural Economics at Washington, D. C., as being representative of a type of decay found responsible for considerable damage to a carlot shipped from St. George, S. C., June 2, 1922. Each fruit was entirely encased in a luxuriant cottony mycelial weft, matted down here and there as a wet membranous layer, at first sight thus suggesting being wrapped in absorbent cotton that had become moistened in places. The tissue in the interior was found very watery and of a peculiar texture, greatly softened, and so lacking in mechanical firmness as to be divided very readily with blunt instruments. Where not occupied by secondary bacterial invaders, the juices draining copiously from the incisions were only slightly turbid. The material gave off a peculiar odor rather inadequately described by the term "marshy"—not pleasant, but having little in common with the putrid smells characteristic of the decay of vegetables due to bacteria.

Since the original discovery of the trouble no additional material has been submitted to the writer, and from inquiry it would appear that the type of deterioration in question is not frequently encountered on the Washington market, or at least not in quantity. However, early in July, 1924, J. I. Lauritzen found several carload lots in both the Pittsburgh and the Buffalo markets, of which not inconsiderable portions were affected in exactly the manner described in the preceding paragraph. Almost simultaneously G. B. Ramsey observed the same decay with its characteristic display of cottony mycelium in a carload lot of cucumbers on the Chicago market, the shipment in this instance having originated in North Carolina. It is highly probable that in the case of the cucumbers grown in the Southeastern States the destruction from this trouble will generally be found greatest in the markets of our more remote northern cities, since, other things being equal, the quantity of cucumbers affected evidently increases with the length of time the shipment is in transit.

Microscopic examination of the specimens obtained on the Washington market revealed the fresh cottony growth as a mass of mycelium composed of nonseptate hyphae. Where the weft had been matted down as a wet membranous layer closely adhering to the substratum, innumerable thou-

[1] Received for publication, Aug. 20, 1924; issued August, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 1042.

sands of oogonia with antheridia and oospores, were found in all stages of development, the entire apparatus being readily recognizable as characteristic of the genus Pythium. The softened tissue was everywhere occupied by branching mycelium, the elements of which showed little evidence of definite orientation (fig. 1). At the points where the hyphae passed through the cell walls they were constricted to approximately half their normal diameter.

Pure cultures of the fungus were readily obtained by placing pieces of diseased tissue on corn-meal agar plates, and transferring portions of

FIG. 1.—Section of cucumber affected with cottony leak, showing tissue occupied by abundance of branching hyphae, and constriction of latter where passing through host cell wall. × 250

mycelium from the margins of the resulting growth to tubes of sterile media. Through the courtesy of J. I. Lauritzen and G. B. Ramsey, transfers of the fungus isolated by them from the diseased material found in Pittsburgh and in Chicago, respectively, were also procured. In general appearance the cultures thus obtained were practically indistinguishable from cultures of the damping-off fungus, *Pythium debaryanum* Hesse. A minor but not insignificant difference could usually be made out in watching the development of the two types of parasites in parallel cultures, as under suitable conditions the cucumber fungus shows development of aerial mycelium in quantity by the end of the second day, whereas in cultures of the damping-off organism such development generally fails to ensue until the third day.

SOME MORPHOLOGICAL FEATURES

Zoosporangia of the fungus from cucumber fruits are readily obtained by putting pieces of invaded cucumber tissue (watermelon or squash tissue occupied by the parasite serve equally well), or thin slices from the surface of Lima-bean agar cultures, into a shallow layer of sterile water, which should preferably be renewed several times to wash away soluble staling products and excessive food materials. In the course of 2 to 5 hours an abundance of new structures are proliferated from the surface and periphery of the old mycelia, consisting of stout axial elements bearing swollen digitate and short diverticulate branches, these branches frequently undergoing close successive ramification to yield somewhat involved complexes corresponding to the structure discussed by Butler (*3*) as "budlike lateral processes." At other times the branches are fewer in number and at irregular intervals in open racemose arrangement. In any case, if the entire apparatus is well developed a number of septa varying from one or several to a dozen are inserted, thus bringing about the delimitation of a variable number of units, each of which may consist, for example, of a digitate branch with its secondary lobulate branches, or of a portion of the axial element with perhaps one or more diverticulate or branching laterals. After pronounced vacuolization of the protoplasm usual

for the sporangia of Pythium, and the development of an evacuation tube from the tip of one of the digitate elements, the contents of each unit escape to form a vesicle in which the zoospores are fashioned. The latter usually vary from 30 to 40 in number, but individual vesicles developing as few as half a dozen or as many as 60 are not rare. Under favorable conditions zoospore production is extraordinarily abundant, the amount of material that can conveniently be accommodated in a 10 cm. Petri dish giving rise to numbers estimated in excess of 100,000 in the course of an hour.

The organism evidently corresponds to a fungus apparently first noted in the literature as a variety of *Pythium gracile* Schenk by Butler (*3*), who in India found it parasitic on roots and base of stem of ginger (*Zingiber officinale* Rosc.) as well as on the roots of castor bean (*Ricinus communis* L.). Later, Subramaniam (*14*) investigated what he regarded as the same form more closely and set it off as a new species, *Pythium butleri*. In the meantime it had been encountered in the United States as the cause of a disease of radishes (*Raphanus sativus* L.) and sugar beets (*Beta vulgaris* L.) by Edson (*6*), who described it as *Rheosporangium aphanidermatum*, the type of a new genus of Saprolegniaceae. The similarity and apparent identity of the American and Indian forms were pointed out by Carpenter (*4*), who found the fungus associated especially with a destructive root rot of sugar cane (*Saccharum officinarum* L.) in Hawaii. More recently Fitzpatrick (*7*) made Carpenter's inferences effective in a nomenclatorial sense by combining Edson's specific name with both generic names, Pythium and Nematosporangium, the resulting binomials being presented as alternatives, choice between which was made dependent on the advisability of retaining or abandoning Schroeter's genus Nematosporangium as distinct from Pythium.

The genus Nematosporangium as defined by Schroeter (*13, p. 104*) was intended to include the forms having sporangia represented by filaments not differing from the vegetative hyphae, as contrasted with the forms possessing subspherical sporangia, which were to be retained in the genus Pythium. The distinction thus drawn is quite similar to that made by Butler (*3*), whose subgenera Aphragmium and Sphaerosporangium correspond closely to the genera recognized in the "Pflanzenfamilien." Recognition of the subspherical sporangium as a common characteristic of one group of forms is supported by excellent morphological evidence. However, the general view that the sporangia of the remaining forms consist of a simple or branching filament, analogous, for example, to the sporangium of Aphanomyces among the Saprolegniaceae, would appear to be in need of drastic revision. The sporangia characteristic of the parasite causing the cucumber decay discussed in this paper are represented, as has been pointed out, by units resulting from the septation of conspicuously swollen elements, corresponding to the structures which Ward (*15*) first figured and described in his account of a fungus he designated as *Pythium gracile* De Bary, and which later Butler discovered in all the members of the subgenus Aphragmium examined by him. Neither of these authors appears to have observed the participation of these structures in the formation of zoospores, Ward supposing them to serve as reservoirs of protoplasm for mycelial growth or the development of oogonia, while Butler assigned to them a probable capacity for surviving unfavorable conditions. In their studies of what presumably were forms identical with the one attacking cucumbers, Edson, Subramaniam, and Carpenter illustrated and discussed the same type of structures as "presporangia," "buds," and "sporangia," respectively, although perhaps without observing them in their most luxuriant development. It may be mentioned that even more distinctive development of this lobulate type of sporangium is exemplified in one of the two species with spiny oogonia found parasitic on watermelon fruits, the larger examples here being represented by a mulberrylike aggregation consisting frequently of more than a score of subglobose communicating elements, from which the contents are delivered through an evacuation tube into a vesicle giving rise to more than a hundred zoospores. The other spiny form (*5*) associated with decay of watermelons exhibits sporangia that may be regarded as a modification of the subspherical type, consisting generally of a subspherical part together with an adjacent part of one or both hyphal elements between which it is intercalated, the evacuation tube arising from the venterlike part, or from the filamentous part, or very frequently from near the juncture of the two. Because Schroeter's disposition makes no provision either for this transitional type of sporangium or for the distinctive lobulate type, his scheme to be usable

would seem to require modification either by appropriately amending the two genera recognized by him or including one or more additional genera. For the present, therefore, it seems best to retain the genus Pythium in its more inclusive sense, as employed in the writings of DeBary and Butler.

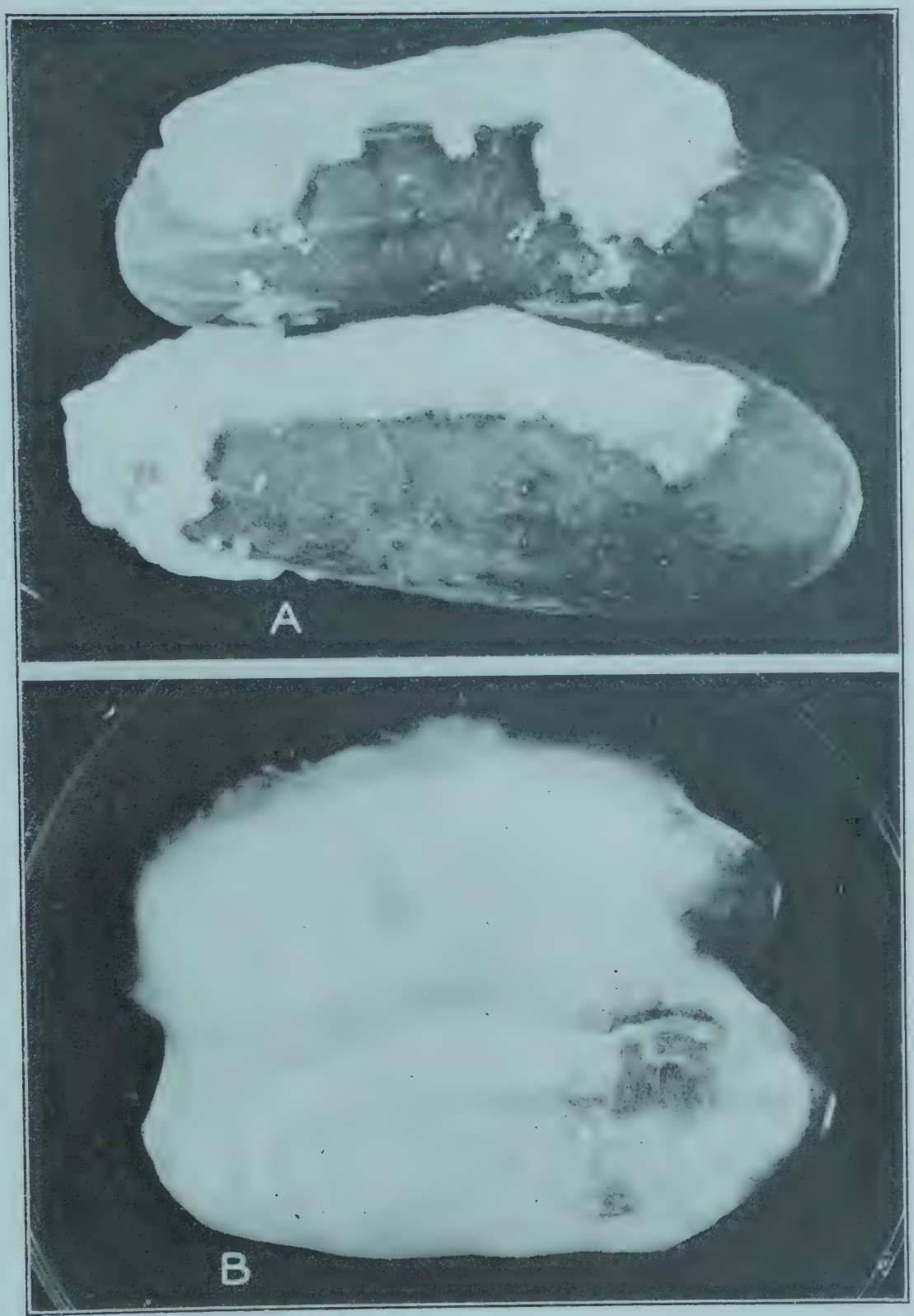
PATHOGENICITY

The pathogenicity of the three strains isolated from material obtained on the markets of Washington, Pittsburgh, and Chicago was repeatedly demonstrated by inoculation into healthy cucumber fruits. Pieces of mycelium from pure cultures were inserted into aseptic incisions, which then were sealed with sterile vaseline, and the cucumbers placed in glass chambers without additional water. Softening, involving the tissues usually for a radius of several centimeters, was manifest within 24 hours; in 48 hours the larger part of the cucumber was involved and aerial mycelium was present in quantity near the point of inoculation, while farther away it appeared in numerous small patches and minute white flecks that marked individual spots where the vigorous crowded hyphae had burst through the confining epidermis (pl. 1, A). At the end of the third day the whole fruit was frequently entirely clothed in cottony mycelium (pl. 1, B). That we are not dealing here with a specialized parasite became evident when altogether similar results were obtained by the use of strains morphologically identical with those derived from the cucumber but isolated from other sources: (1) From dead female nematodes, *Heterodera radicicola* (Greef.) Müller, in material supplied by N. A. Cobb and G. Steiner, where the occurrence of the fungus as a saprophyte or a possible parasite invading moribund specimens could not be clearly determined; (2) from pea (*Pisum sativum* L.) roots exhibiting symptoms of root-rot; and (3) from watermelon fruits affected with the buff blossom-end rot.

The cucumber parasite was tried out on a number of other economic cucurbitaceous fruits. As might be expected, watermelons were found highly susceptible to attack, the resulting decay being entirely similar to the buff blossom-end rot familiar to the writer as a field trouble apparently widely distributed in the Middle Atlantic States, and for the most part due to the identical fungus. Pattypan, vegetable marrow, and summer crookneck squashes, all representing varieties of *Cucurbita pepo* L., are as promptly attacked and destroyed as the cucumber and with the same luxuriant development of extramatrical mycelium (pl. 2, A, B). Experiments with muskmelons (*Cucumis melo* L.) have not been quite as satisfactory, owing to the difficulty of avoiding bacterial contamination, especially in riper specimens. In general, it appears that in the green condition in which this fruit is frequently found on the market, the muskmelon does not provide a substratum very suitable for the fungus, but that as maturity is approached the soft edible pulp is more readily invaded. It is possible that the fungus participates in the destruction of rejected muskmelons left in the field; a considerable portion of the abandoned muskmelons in some Delaware fields visited by the writer in 1922 exhibited, as the initial stage in decomposition, a very watery condition of the interior, associated with the peculiar marshy odor fairly presumptive of the presence of some species of Pythium.

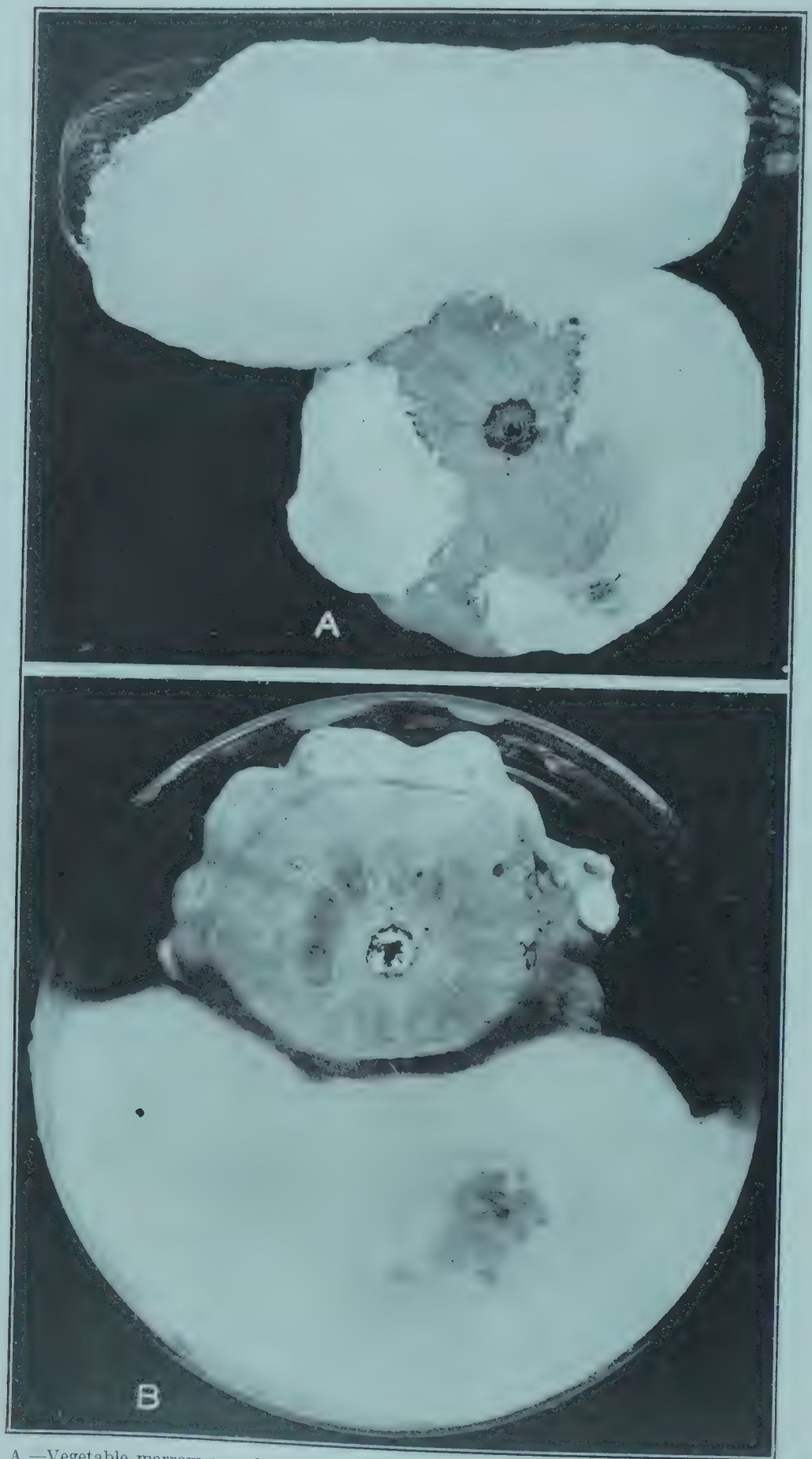
When inoculated under the rind of honeydew melons and cassaba melons (*Cucumis melo* L.), the fungus is able to establish itself, but subsequent development is markedly slow, sometimes being scarcely one-tenth as rapid as in cucumbers. The mycelium found in the tissues is of a compact, densely branching type, similar to that obtained on artificial media excessively rich in food materials, indicating that the juices of these fruits are too concentrated to permit of normal growth. Several inoculations into the flesh of Hubbard squash (*Cucurbita maxima* Duchesne) failed to result even in incipient infections, although the possibility that this vegetable is amenable to attack under other and more favorable conditions is not be to excluded.

While decay of cucumbers in transit has hitherto been found associated with only one species of Pythium, this is not because cucumbers are resistant to congeneric forms. In the course of routine procedure for obtaining the production of zoospores, for which purpose the tissue of cucurbitaceous fruits is not without merit, the writer has inoculated cucumbers with scores of strains having smooth oogonia, subspherical sporangia (or conidia), and fluffy aerial mycelium, belonging evidently to a number of related species—in short, with strains of the type traditionally and no doubt often correctly designated in papers on plant diseases as *Pythium debaryanum* Hesse. These strains have been isolated, for example, from the



A.—Two cucumbers 45 hours after inoculation with pure culture of strain of *Pythium aphanidermatum* isolated from diseased cucumber collected from carload lot at Washington, D. C., June, 1922. × ½

B.—Same two cucumbers as in A, but 24 hours later. × ⅖



A.—Vegetable marrow squash and pattypan squash 60 hours after inoculation at three points with pure culture of Washington strain of *Pythium aphanidermatum* isolated from diseased cucumber. × ½

B.—Three pattypan squashes 72 hours after inoculation with strain of *P. aphanidermatum* isolated from diseased cucumbers at Chicago, 1924. The profuse cottony mycelium in the lower part of figure has completely invested the two younger and more tender specimens. × ½

stems of cucumber plants affected with the trouble described by Atkinson (*1*) as canker; from diseased roots of herbaceous hosts, including garden peas, sweet peas (*Lathyrus odoratus* L.), rhubarb (*Rheum rhaponticum* L.), sweet potatoes (*Ipomoea batatas* Poir), cress (*Lepidium sativum* L.), and spinach (*Spinacea oleracea* L.); from roots of diseased seedlings of woody plants like *Pinus ponderosa* Dougl., *P. banksiana* Lamb., *P. sylvestris* L., *P. aristata* Engelm., and *Picea sitchensis* Trautv.; and from rose, pear, and geranium cuttings which had become diseased after being put in propagation beds. With relatively infrequent exceptions, the inoculated cucumber was attacked and the tissue invaded in much the same way as when *Pythium aphanidermatum* was employed, the rate of destruction for some forms being about equally rapid, while in the case of others advance was slower. All of the strains of Pythium isolated from separate lots of "leaky" potatoes and made available to the writer through the courtesy of G. K. K. Link have proved uniformly destructive, as have also about a dozen similar strains of the *P. debaryanum* type isolated from watermelons affected with a decay not readily distinguishable from the buff blossom end-rot due to *P. aphanidermatum*.

Although the several species of Pythium with subspherical sporangia (or conidia) and smooth oogonia show certain minor differences, in that some bring about greater softness in affected tissues, or a more watery condition than others, their effect in the interior of cucumber fruit is markedly similar to that produced by the parasite isolated from naturally infected material. The much more profuse development of aerial mycelium nevertheless provides a conspicuous characteristic by which attack by *Pythium aphanidermatum* can be distinguished from attack by the congeneric species that have been tried out. This feature appears sufficiently distinctive to merit attention in considering a common name for the disease under consideration. The term "cottony leak," descriptive of the most obvious symptoms of the malady, is proposed in this connection.

In its copious extramatrical development, moreover, is apparently to be found the characteristic to which *Pythium aphanidermatum* owes much of its destructiveness to cucumbers when packed as in commercial containers. The aerial mycelium of an individual fruit bearing an original infection grows out between adjacent fruits, partially or completely investing them. Laboratory experiments leave no room for doubt that such investment results in the infection of cucumbers, immediately if the epidermis is wounded, but without any considerable delay even if the epidermis is, as far as can be ascertained, altogether free of wounds. With the infection communicated from one fruit to another, each infected specimen gives rise in the course of 5 to 10 days to a "nest" of decaying cucumbers, including perhaps from a dozen to a score of individuals. Other species of Pythium with relatively feeble extramatrical development under conditions of only moderate humidity, such as generally prevail in produce cars, fail of passage from fruit to fruit, at least within reasonable periods of time. It is thus possible that if losses due to such species occur, the restriction of infection to single individuals might have occasioned their being overlooked by inspectors and others concerned in the examination and handling of food products.

In addition to cucumbers, the patty-pan squash, the summer crookneck squash, as well as the more delicate-skinned specimens of vegetable-marrow squash, have proved subject to infection by contact or investment with extramatrical mycelium of *Pythium aphanidermatum*. In cucurbitaceous fruits having a rind of indurated tissue like the watermelon, the cassaba, the honeydew melon, and the muskmelon, attempts at inoculation by means of surface contact have not given positive results.

It has been mentioned that some of the forms usually assigned to *Pythium debaryanum*, comprising, however, a relatively small minority, have failed to attack cucumbers when inoculated into incisions. A species not yet identified, provided with lobulate sporangia and hence closely related to but not identical with *P. aphanidermatum*, which was isolated from diseased corn roots, has shown no evidence of pathogenicity on cucumber fruit. The same statement holds true also of the two species with spiny oogonia (Artotrogus) responsible for the widespread chocolate blossom-end decay of watermelons, strains of these forms isolated from fruit thus affected as well as from pear cuttings, sweet-potato rootlets, and pea rootlets proving equally ineffective in producing decay of cucumbers. A third spiny species, in which the considerably larger oogonia are regularly borne on lateral branches, the swollen, somewhat contorted distal portion of which apparently serves as an intercalary antheridium, isolated only once from moribund rhubarb buds, similarly proved innocu-

ous to cucumbers. After securing negative results with three spiny forms, the writer was interested to discover that a fourth form derived from pea roots affected with the root-rot due to *Aphanomyces euteiches* Dr., and apparently different from the other three, attacked cucumbers with moderate vigor, the tissues becoming soft and watery.

In considering means of controlling losses from cottony leak, it is unfortunate that no information is available concerning the incidence of original infections. Knowledge as to whether such infections take place in the field or subsequent to picking would appear to be of primary importance. As the progress of the parasite at lower temperatures is relatively slow, and extramatrical development is reduced to small proportions in the absence of water of condensation and high humidity, attention to proper ventilation combined where practicable with refrigeration might be expected to check the spread of the infection to stock in good condition at the time it was packed.

The attention of students of plant diseases is directed to the very evident partiality of species of Pythium for the fruit of many Cucurbitaceae. Losses in the field due to their parasitism is undoubtedly more considerable than the paucity of references in the literature might lead one to suppose. Parisi's record (*12*) of the occurrence of *P. debaryanum* on the fruit of chayote (*Sechium edule* Sw.) in the botanical garden at Naples in December, 1920, is pertinent in this connection. Not less interesting is the very recent report by McRae (*10*) of the association of strains of Pythium with the decay of *Luffa acutangula* Roxb., *L. aegyptiaca* Mill., *Trichosanthes anguina* L., and *Lagenaria vulgaris* Ser. in India, where these members of the Cucurbitaceae are grown as vegetables.

SUMMARY

Cucumbers grown in the Southeastern States have, on arrival at the northern markets, shown occasional losses due to a disease for which the term "cottony leak" is proposed. It is caused by a species of Pythium identified as *Pythium aphanidermatum* (Eds.) Fitz., the infection being communicated from diseased fruits to adjacent healthy ones by copious production of extramatrical mycelium.

The fungus is strongly parasitic on watermelons, on which host it is responsible for one of the blossom-end rots widely prevalent in the Middle Atlantic States. On inoculation it is rapidly destructive to patty-pan, vegetable-marrow, and summer crookneck squashes.

The sorting out of cucumbers harboring the fungus and the lowering of humidity and temperature by adequate ventilation, combined possibly with refrigeration, are indicated as means for controlling the trouble.

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COMPARATIVE STUDIES OF PYTHIUM DEBARYANUM AND TWO RELATED SPECIES FROM GERANIUM [1]

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INTRODUCTION

Stem rots of geranium cuttings (Pelargonium spp.) have been frequently reported in the literature and ascribed to various microorganisms. Ward (*20*) [2] cultured *Pythium debaryanum* on live Pelargonium cuttings in the course of his classic studies with this fungus. Prillieux and Delacroix (*18*) described *Bacillus caulivorus* as the causal agent of a stem "gangrene" of Pelargonium, potato, clematis, and begonia, and reported *B. pyocyanus* on Pelargonium from Germany. Galloway (*12*) noted a stem discoloration, accompanied by shrivelling and softening, which extended upward from the severed end until the whole stem was involved. Many bacteria but no fungi were observed filling the cells of the advancing area of discoloration. The disease was considered possibly identical with that of Prillieux and Delacroix, which was also later described by Chifflot in a résumé of geranium diseases (*9*). This writer also described a Pelargonium stem rot due to Botrytis sp. Peters (*17*) obtained *P. debaryanum* from diseased geranium stems, which are well illustrated in a colored plate showing typical progressive blackening of the cuttings. He made successful pure culture inoculations and also reproduced the disease by placing healthy cuttings in soil infested with this organism. Inoculation usually resulted in early death; infected cuttings seldom remained partially diseased. Johnson (*16*) isolated this organism from blackened geranium cuttings. Buddin and Wakefield (*5*) obtained *P. debaryanum*, as well as *Botrytis cinerea*, on geranium stems.

The writer has had four species of Pythium under observation since 1919, isolated from diseased geranium cuttings. One of these, *Pythium complectens*, n. sp., was described in a previous paper (*4*). The second (labeled *B* throughout this study) corresponds morphologically to *P. debaryanum* as described by Hesse (*15*), De Bary (*1*, *2*, *3*), Ward (*20*), Butler (*6*, *7*), and others who have worked with and defined the morphological characters of this fungus. The remaining two, which have also proved pathogenic in pure culture inoculation experiments, differ morphologically and physiologically from *P. debaryanum* but are clearly related to it. The present paper is a report of a comparative study of organism *B* and the two related fungi, which have not been found to be wholly identical with any previously described Pythium species.

THE DISEASE

SIGNS

The symptoms of disease caused by any of the Pythium species here described consist of a progressive blackening, shrivelling, and necrosis, starting at the base of the cutting and rapidly involving the entire stem and the petioles (pl. 1). The leaves wilt down when the petioles are reached and death of the entire plant soon ensues. The pith is hollowed out by a soft, wet rot. Epidermal cylinders (except in very young and succulent cuttings) and fibrovascular bundles are not attacked except in the late stages, when secondary organisms enter into consideration. Little difference in the character of the lesions caused by any of these fungi can be observed except in the rate of progress, which is similar for *B* [3] (*P. debaryanum*) and *A*, but distinctly less rapid for *D*. The complete destruction of the cutting is in marked contrast with the stoppage of infection previously described for *P.*

[1] Received for publication July 29, 1924; issued August, 1925.

[2] Reference is made by number (italic) to "Literature cited," 1062.

[3] For the sake of brevity the isolations were lettered *A* to *D* in their order of isolation, and will be so designated in this paper as far as the section in which their taxonomy is considered; *B* referring to *P. debaryanum*, *C* to *P. complectens*, *A* to the large conidial Pythium, and *D* to the form producing the characteristically irregular, swollen, asexual fruiting bodies.

complectens, where a cork layer laid down in advance of infection prevents further progress of the hyphae after nfection has already extended some distance from the point of inoculation. The rate of progress of infection is lways less rapid where comparatively mature plants are involved (pl. 2), and may be localized when the epidermal cylinder is thickly cutinized and the basal tissues are old and woody.

The hyaline, coenocytic hyphae of these fungi may be observed in and between cells of the less discolored advancing area, particularly as long strands along the fibrovascular system (pl. 3, A). Farther back the fruiting bodies are formed, including oospores and conidia in the case of *B* (*P. debaryanum*); conidia only were formed in tissues by the other two Pythium spp. Bacteria were frequently observed in the more decayed tissues, particularly in xylem cells, but not in the less infected regions where advancing hyphae could be seen.

ISOLATION

P. debaryanum (*B*) and the large conidia form *A* were first isolated, occurring together in a plate colony made from a single diseased geranium from the Washington greenhouses. They were separated by making single-spore cultures from conidia, those of *A* being by far the larger and readily recognizable. Form *D* was obtained alone in a later isolation. Repeated isolations from naturally blackened and diseased geranium cuttings in the Department of Agriculture greenhouses have yielded these fungi, occurring sometimes together, usually singly. The methods of isolation used have been described in the paper on *P. complectens* (*4*). Cultural studies from single-spore isolations soon showed that these fungi are not identical, nor corresponding to previously described species of Pythium with the exception of *B*, the *P. debaryanum* isolation.

PATHOGENICITY

Controlled inoculation experiments involving some 230 cuttings in 17 inoculation series since isolation have shown that the disease can be reproduced with great uniformity when the inoculum is placed at the cut base of fresh Pelargonium cuttings in sterilized soil, using any of the three isolations. Progressive discoloration and rot occurred as in naturally infected cuttings from the severed end up; check plants formed a callus as a rule and rooted normally. No differences in the symptoms produced could be observed with either isolation, except in the rate of progress; *Pythium debaryanum* (*B*) and isolation *A* were the most severe. Death ensued within 6 to 10 days, depending on the organism used. Material fixed in Flemming's strong solution and in Merckel's fluid and sectioned showed the hyphae within the tissues, conidia in the case of *A*, conidia and oospores in the case of *B*. Each was readily reisolated from advancing margins of the discoloration. Repeated reisolations with subsequent inoculation have shown their ability to attack geranium cuttings. Cross-inoculations on coleus, begonia, cucumber seedlings, and radish seedlings result in soft rots in the first two hosts and typical damping off in the seedlings, form *B* causing by far the most severe infection. *A* and *D* required a longer incubation period in the case of the seedlings and caused slower progress of the disease. In one experiment where cuttings were placed in soil which had previously contained plants artificially infected with Pythium species, four out of five were infected where *B* had been used, two were killed and three showed basal blackening when *A* had been used, and four out of five showed infection when *D* had been used, indicating the rôle of the soil as a carrier. After four or more years in culture, there is a distinct reduction of virulence evident in a slower progress of discoloration and lower percentages of infection compared with the uniformly complete infections obtained early in the work.

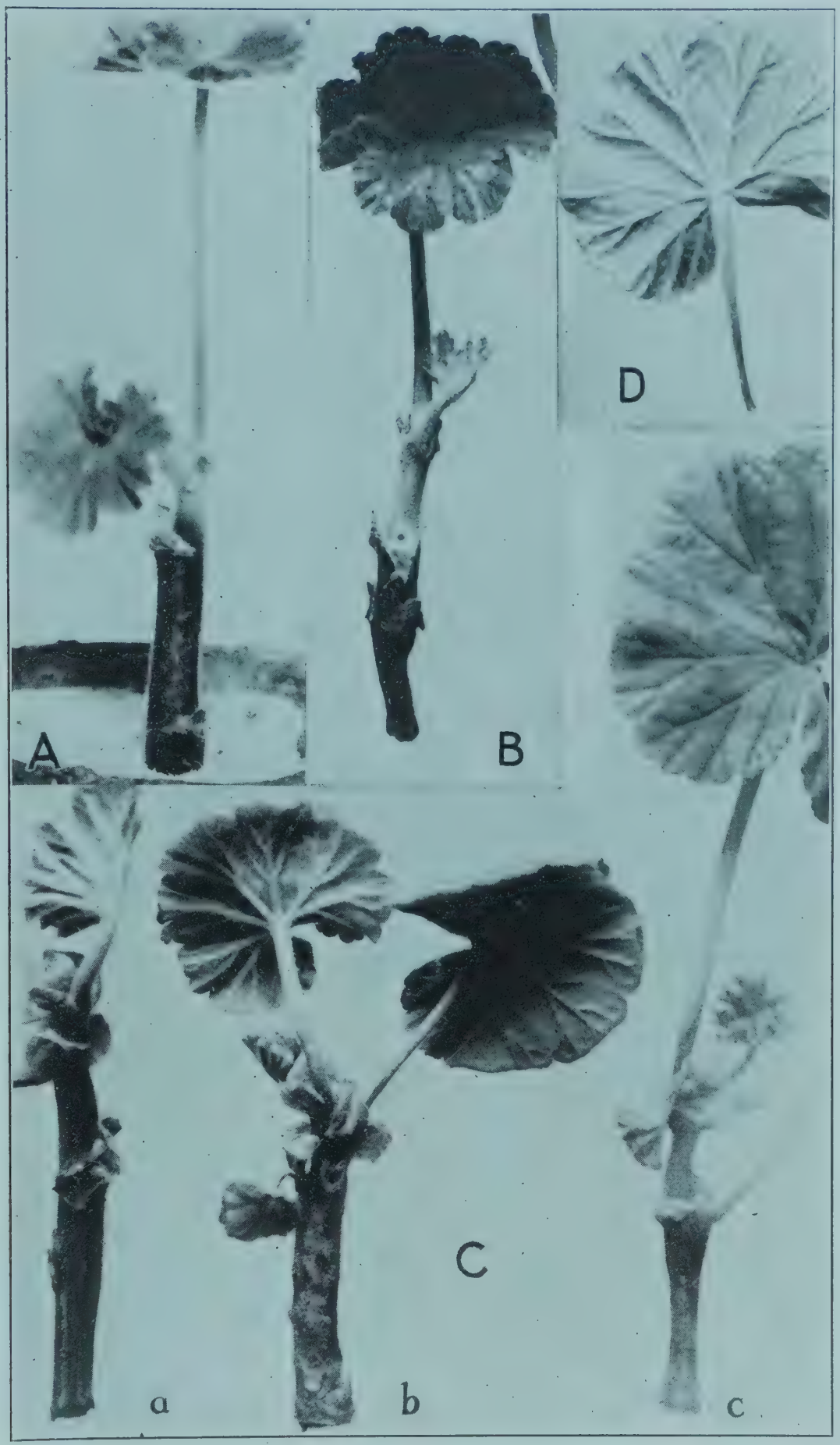
The comparative rates of discoloration caused by each of the organisms are shown below in data from one of the inoculation experiments (September 15, 1921):

ISOLATION A.—Ten plants inoculated at the base. Discoloration averaged 35.9 mm. up the stem by the second day; 59.4 mm. by the fifth day, with five plants collapsed. Eight were dead the sixth day, and the discoloration on the remainder averaged 67.2 mm.

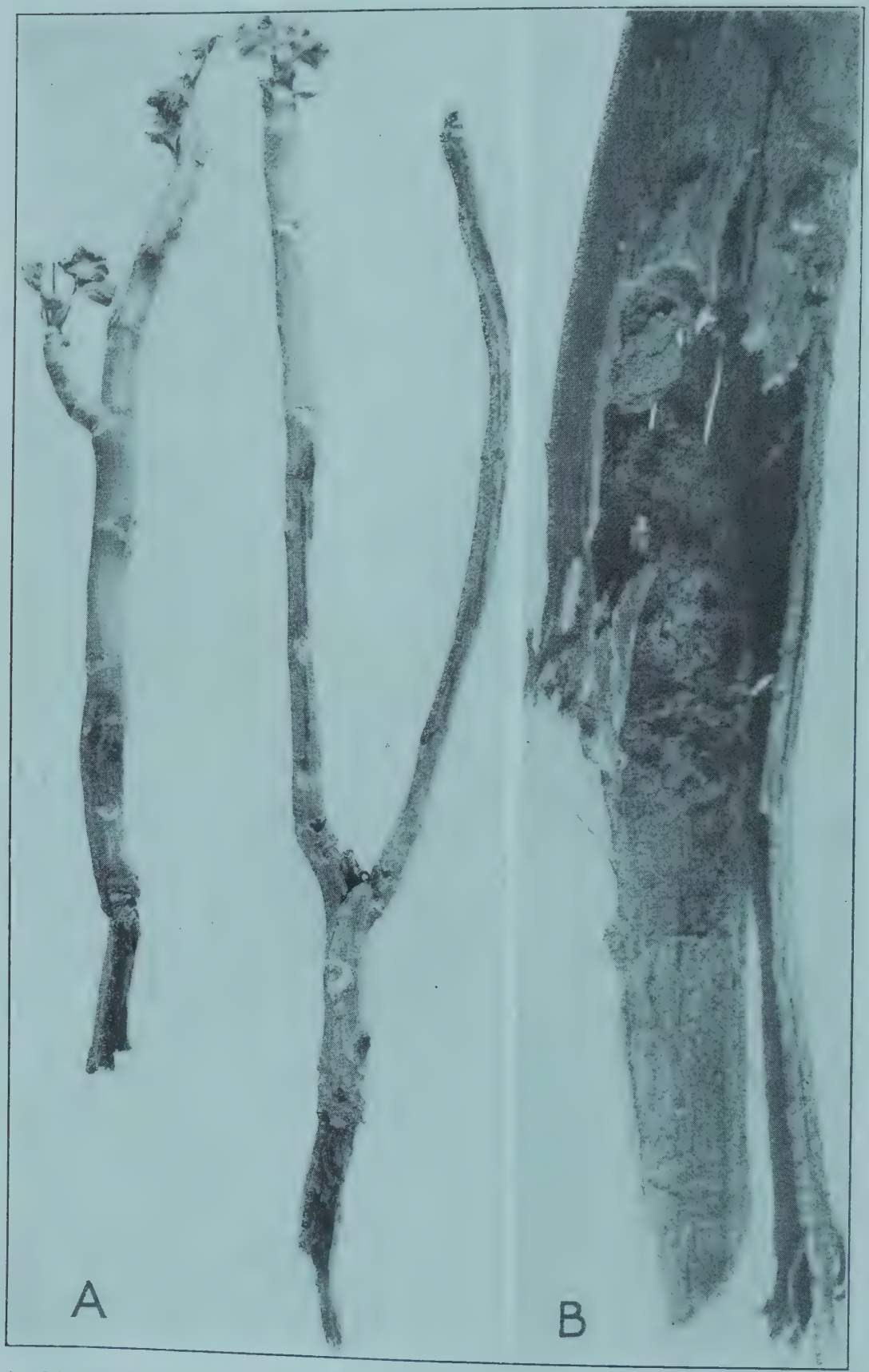
ISOLATION B.—Ten plants. Discoloration reached an average of 32.6 mm. up the stem by the second day; eight had collapsed by the fifth day, the remainder averaging 63.7 mm. All were dead and on the ground the sixth day.

ISOLATION D.—Ten plants. Discoloration reached an average of 21.6 mm. the second day, 32.7 the fifth day, 38.5 the sixth day. None were dead till the tenth day. All had collapsed four days later.

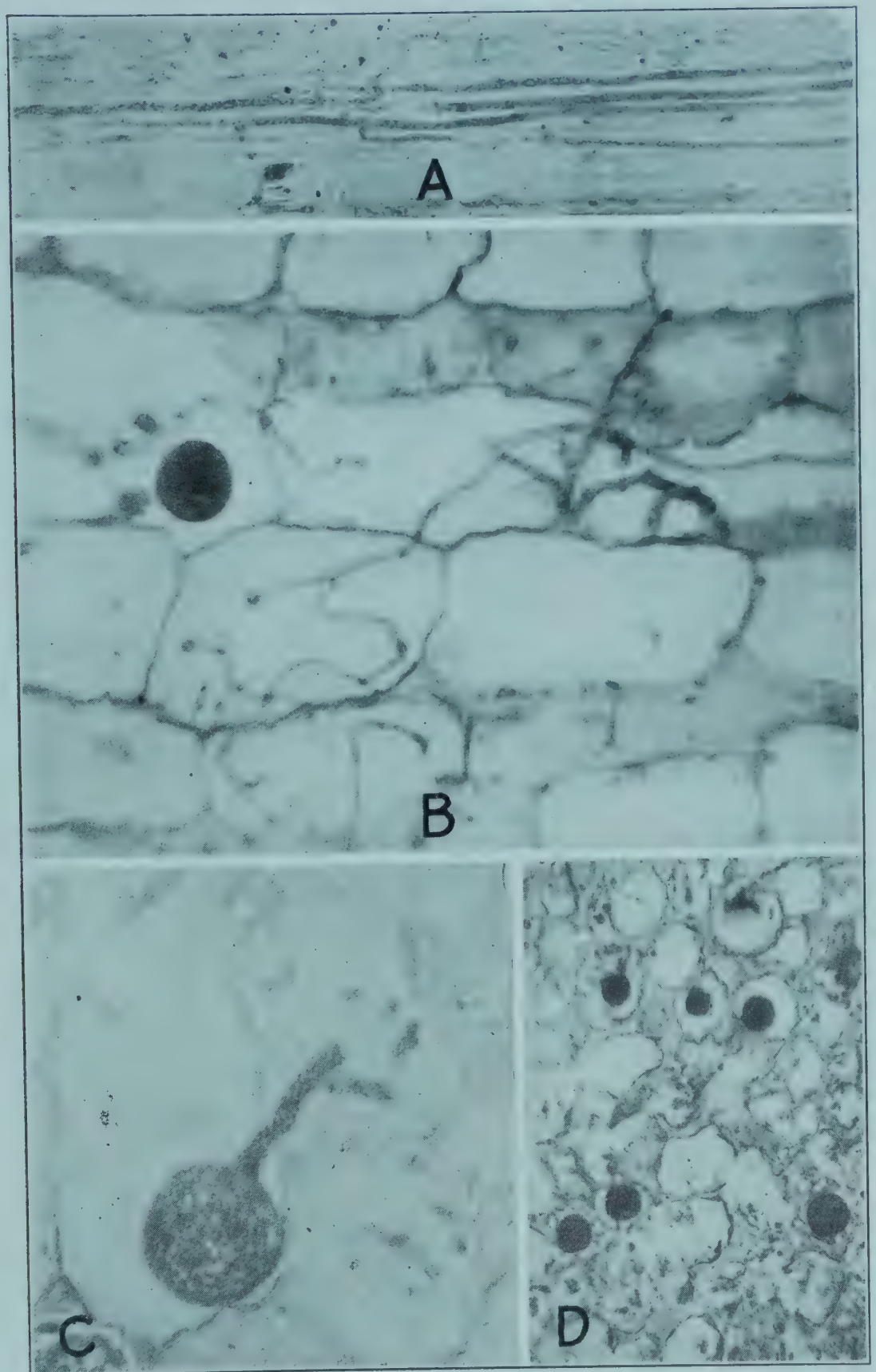
Ten checks remained healthy, formed a callus and rooted long before infected plants began to collapse.



A.—Naturally infected geranium cutting; *P. debaryanum* was isolated
B.—Cutting naturally infected with *P. debaryanum* var. *Pelargonii*, Washington greenhouses
C.—Artificial inoculations, fifth day; Ca, *P. debaryanum;* Cb, *P. splendens;* Cc, *P. debaryanum* var. *Pelargonii*
D.—Leaf from Cb, showing the shriveling and discoloration of the base of the petiole



A.—Mature plants from Enid, Okla., from which *P. complectens* was isolated (1923)
B.—Lower part of stem right, enlarged nearly five times. The rotting away of pith, leaving the epidermal cylinder intact for some time, was characteristic of each of the organisms studied



A.—Mycelium of *P. splendens*, running between companion cells in long strands
B.—Mycelium of same, in pith cells; also conidium
C.—Conidium being formed in pith cell. Note constriction of hyphae through cell wall
D.—Conidia sprinkled through the pith

PATHOLOGICAL HISTOLOGY

The hyphae are both intercellular and intracellular, and may be observed in all tissues except the fibrovascular long before the cells collapse. Constriction at passage through the cell wall is the rule (pl. 3, C). Long strands run between the companion cells, branching out at intervals into the pith and cortex. Here the development of the mycelium is profuse, often forming coiled nests within cells and branching abundantly and irregularly. The host protoplasm is rapidly disorganized; but nuclei and starch grains are still recognizable even in late stages of tissue disintegration.

The large conidia of *A* are formed abundantly within the cells, sometimes in the intercellular spaces. In the latter case and when formed in the elongated companion cells or xylem they may be flattened out or compressed in accordance with the shape of the surrounding host cell walls. Two or three conidia may be found in a large pith cell. Oospores of *A* have not been observed within the tissues; *P. debaryanum* (*B*), however, forms them in abundance, as well as conidia, though to a lesser extent. No oospores of *D* were found within infected tissues. This organism forms round conidia to a small extent; nests of hyphae, irregularly swollen places in the mycelium and profuse branching were more frequently observed. The curious sickle-shaped bodies so abundantly formed in cultures of all three were not found in the tissues.

Discoloration of the host cell walls by oxidation products of the disorganized cell contents is followed by collapse and soft rot accelerated by the action of secondary organisms on the dead cells. The stems of succulent cuttings are rapidly reduced to a black flaccid mass which remains in the soil if the still healthy part is pulled off, and acts as a source of infection.

THE FUNGI

MORPHOLOGY

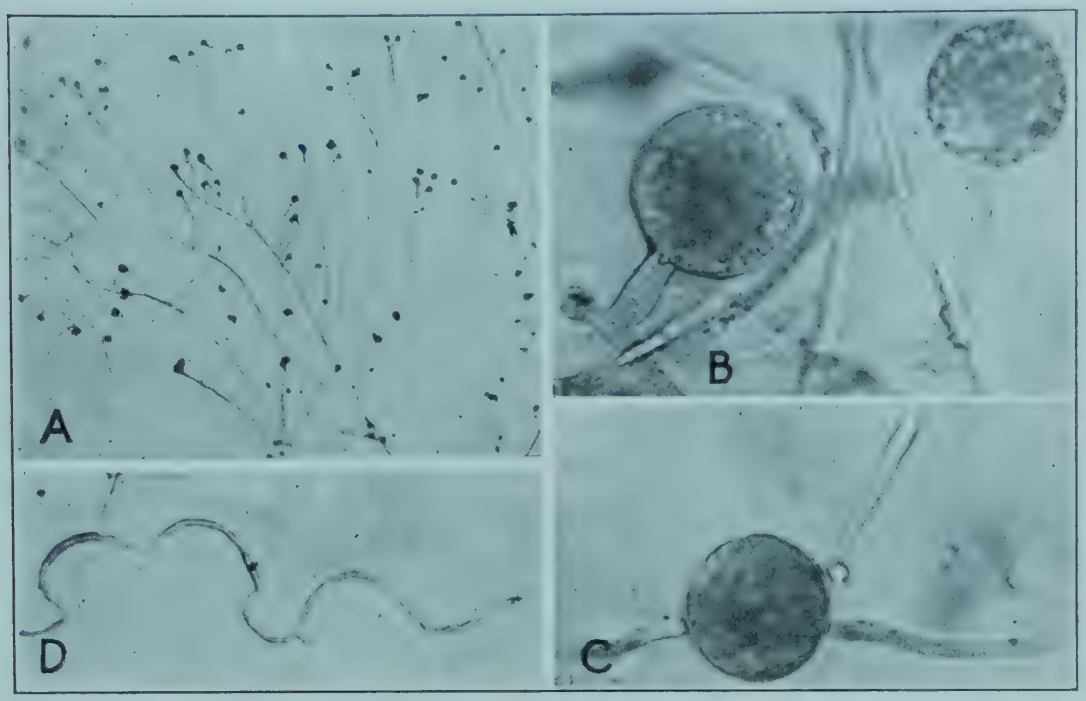
The morphology of *Pythium debaryanum* as it occurred in the writer's cultures will be briefly described, for comparison with the related fungi herein discussed. The hyphae are 3.5 μ to 10.0 μ thick, average 6.4 μ, richly branching from strongly developed main hyphae. The small subsidiary branches curve in all directions, resulting in plate colonies which appear uniform and finely granular to the unaided eye; this type of growth is in sharp contrast with the combed-silk effect characteristic of *P. complectens*, caused by parallel radial growth of the branches. Variations from the normal hyphae are to be found in irregular knotted masses or coils, and in clavate, sickle-shaped bodies abundantly developed within two days of growth on plates (pl. 4, D). Carpenter (*8*) figures similar bodies in *Pythium butleri* from sugar cane (his pl. 16, figs. 1 and 2). In the writer's cultures they were developed at the interface of agar and glass, and near solid particles in the media, indicating a possible appressorial nature. Chains of these clavate bodies, which bore a striking resemblance to antheridia except that they were larger, and not near oogonia, were found in plate cultures of each of the Pythium species under observation.

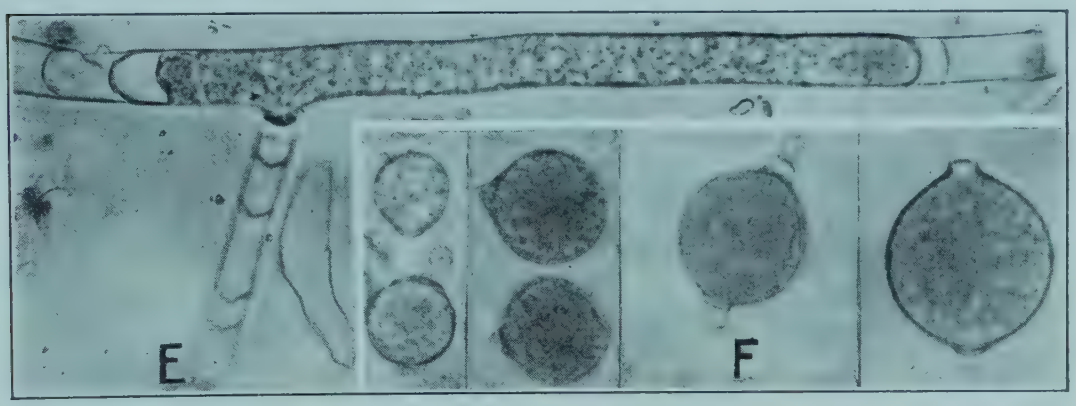
Conidia are smooth-walled, subspherical, terminal, or intercalary, 16.6 μ to 26.2 μ in diameter, averaging 21.7 μ. Germination takes place by germ tubes, usually one. Zoospore formation has not been observed.

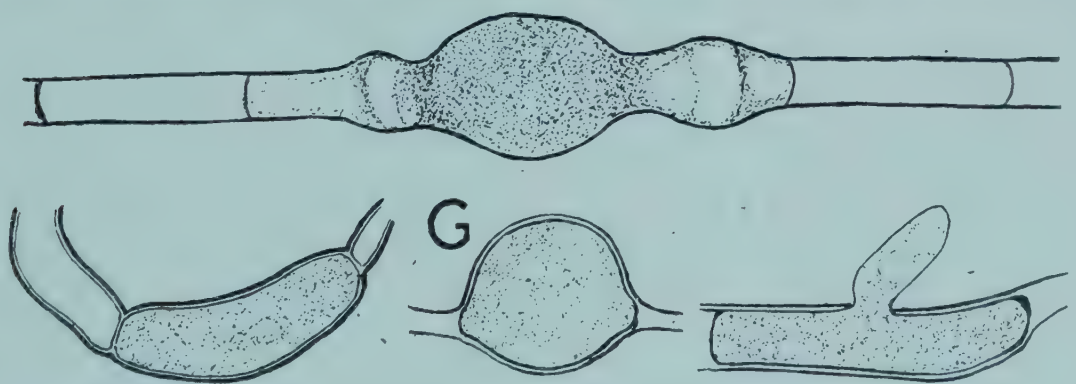
Swollen intercalary bodies are sometimes found in old cultures where the media is drying down. These appear more irregular in size and shape than the conidia formed in the early stage of growth, but also germinate by pushing out a germ tube.

Oospores are found in great abundance on corn meal agar and oatmeal agar and are far more frequent than conidia. The oogonia are 16.8 μ to 25.8 μ in diameter, averaging 25.1 μ; oospores are 14.7 μ to 21.3 μ in diameter, averaging 18.1 μ. The oospores lie free in the oogonia. Both are smooth-walled. Antheridia are clavate, single, less frequently two or three per oogonium; a well-marked fertilization tube is put forth from the tip of the antheridium which is applied to the oogonium wall. Fertilization has been frequently followed through in living material and does not differ essentially from the accounts of De Bary and Ward, except in the inception of the exospore wall, which takes place by the tangential extension of a peripheral disk as described for *P. complectens* (*4*). Germination of the oospores has not been observed.

The fourth Pythium (*D*) resembles the above organism in the size of the hyphae and in the method of branching. Round, smooth conidia are produced in moderation but show greater variation in size, ranging from 12.8 μ to 27.7 μ, average 20.1 μ. The most characteristic feature is the intercalary for-







A.—*Pythium splendens*, conidial formation terminally at ends of short branches. ×12+
B.—Swelling up of tips of conidiophores to form conidia. ×500 circa
C.—Germination of conidia (several germ tubes) in tap water
D.—Sickle-shaped, clavate bodies formed in chains on plates; present in all the forms studied. ×50
E.—Retraction of protoplasm to form the large intercalary body, showing septa successively laid down
F.—Round conidia, *P. debaryanum* var. *Pelargonii*. ×550
G.—Irregular, swollen intercalary bodies characteristic of this organism, also showing germination

mation in plate cultures, within seven days after inoculation, of irregular swollen bodies filled with a dense protoplasm which has withdrawn from adjacent regions of the hyphae, laying down septa as it contracts (pl. 4, E). Such a condition is a not uncommon phenomenon in a variety of old fungus cultures; in this case, however, it is produced quite normally after a few days of growth. The general contraction of the protoplasm into these bodies with abundant septation of the empty hyphae occurs in 7 to 10 days after inoculation of the plates. Such colonies are granular but otherwise almost transparent and without the filamentous structure usually visible to the eye in fungus cultures. Hand-lens inspection reveals a multitude of short straight crystal-like bodies, which are the septa formed in the clear hyphae by the retracting protoplasm. Germination of these resting bodies takes place by a tube, as in the case of the round conidia.

A second characteristic of *D* is the scarcity of oospores, contrasting sharply with the profusion of oospores produced by *B* on plates of nearly all media. None were obtained for 14 months after the original isolation, though 16 media were tried. They finally appeared in hanging drops of sterile oatmeal decoction four days after inoculation, and have since appeared sporadically in cornmeal agar plates. The conditions necessary for oospore formation are not known, aside from the facts that long-continued growth and transfers on artificial media preceded their appearance, and that they are most likely to appear immediately around and on the mycelium used as the inoculum, rather than on the fresh growth.

In measurements the oospores of *D* vary little from those of *B*, ranging from 15.9 μ to 19.9 μ, averaging 17.8 μ. One to four antheridia are present, all emptying their contents. They are usually broader at the apex than are antheridia of *B*, and differ also in that they often lie appressed to the oogonium along their entire length. Contraction of the oosphere and fertilization through a tube occurs as described in this group. Complete maturation is however the exception rather than the rule; the contracted fertilized oosphere often degenerates into a mass of oily globules which later merge into a large vacuole filling the oogonium except for a peripheral layer of hyaline granules, no oospore wall being formed. Oospores that have come to maturity lie free in the oogonium and are smooth walled. The walls are distinctly thinner than those of *B* (pl. 5, C), and are hyaline or yellowish. The contents are granular except for a single eccentric vacuole.

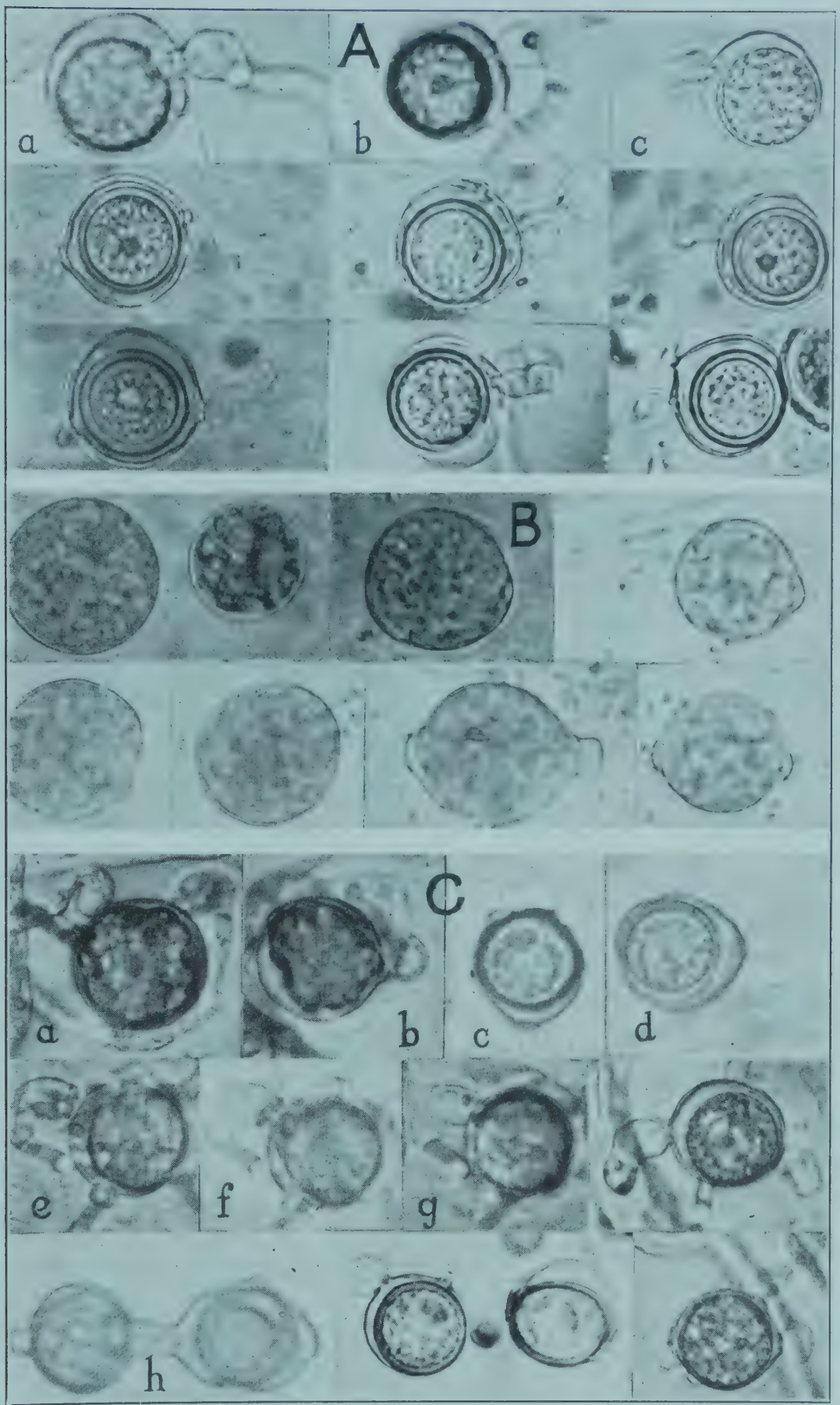
Attempts at germinating the oospores of *D*, including the use of freezing, alternate temperatures, etherization, and soaking in water, have not succeeded. The round conidia and the swollen irregular bodies are clearly the stages on which the organism is dependent for dissemination and the tiding over of unfavorable conditions, since oospores have not been found in nature and are so scarce in culture.

The sickle-shaped bodies characteristic of these Pythium spp. were developed in plate cultures two days after inoculation.

Type *A*, the organism first isolated, is characterized morphologically (1) by the abundant formation of spherical terminal conidia on all media and in the host plant, much larger than those of *B* (21.7 μ to 48.9 μ, average 36.2 μ); (2) by a comparatively small production of large oogonia (25.5 μ to 34.7 μ, average 31.7 μ) and oospores (21.3 μ to 29.8 μ, average 26.6 μ) accompanied regularly by three to eight antheridia. Comparative measurements from this organism and *B* are shown in Figures 1 and 2. Hyphae are 3.5 μ to 9.2 μ wide, average 6.4 μ, and consist of thick main branches with curving lateral branches arising at acute and right angles, constricted at the point of origin. The sickle-shaped bodies are also present, often growing in chains (pl. 4, D).

The conidia appear within two days on culture media, and are formed by the swelling up of a hyphal tip. Intercalary conidia have not been observed, in marked contrast with the abundance of this type in cultures of the other organisms studied. The conidia are smooth and thin walled, and are filled with a densely granular protoplasm which becomes vacuolate with age. Conidia from potato cylinders, beef agar, and potato agar without dextrose are abnormally hyaline and collapse sooner than those from media rich in sugars and readily available food, as sugar-beet agar and oatmeal agar. Drying out of the media in all cases is followed or accompanied by collapse and death of the conidia. Those in the depth of the media, therefore less exposed to drying, are more likely to remain plump and viable, cultures sometimes remaining viable after 10 months.

The conidia of *A* are formed in such abundance on all media that the colonies rapidly assume a finely granu-



A.—Type *B. Pythium debaryanum*, oospores in various stages of fertilization and maturation. ×750
B.—*P. debaryanum*, conidia. ×750
C.—*P. debaryanum* var. *Pelargonii*, oospores. ×750. Fertilization and maturation. Successive stages in fertilization of a single oogone in e-f-g. Note intercalary oogonia, plurality of antheridia, and broad apex of antheridia; also the thin wall of the oospore

lar appearance, and individual conidia may be observed with a low-power hand-lens along the edges of the growth when it has reached the glass sides. Frequently each lateral branch of a hypha becomes a conidiophore off by a septum and drops off. Persistent conidia and germination *in situ* are exceptional. Zoospore formation has not been observed, even when the same environmental conditions are applied which induced their formation

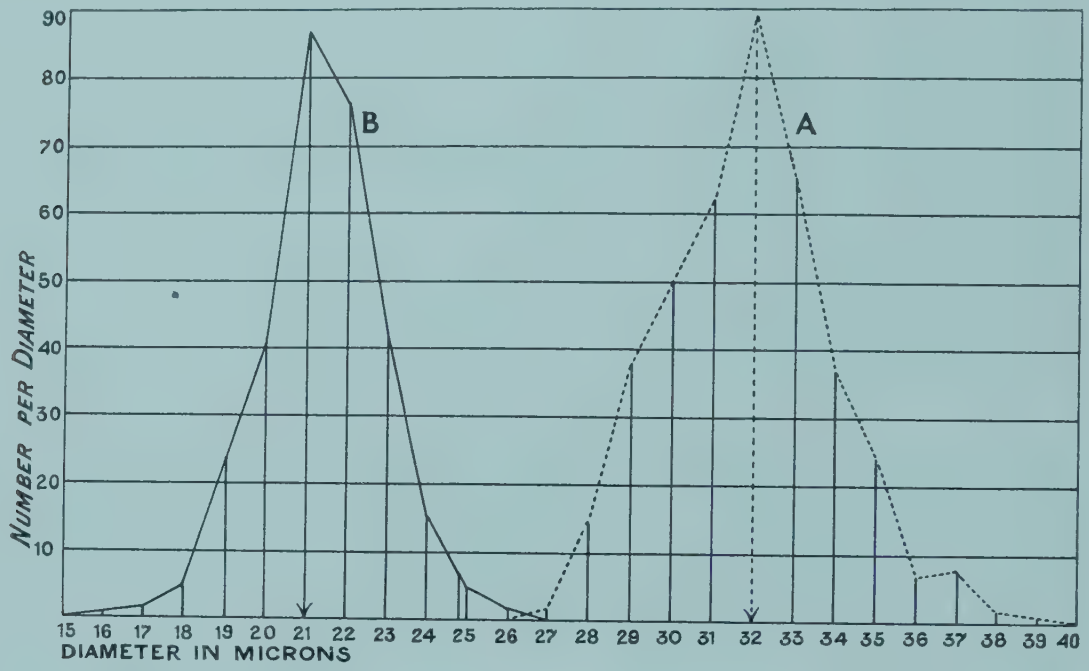


FIG. 1.—Measurements of oogonia of *P. debaryanum* (300) and of *P. splendens* (400) plotted as frequency curve

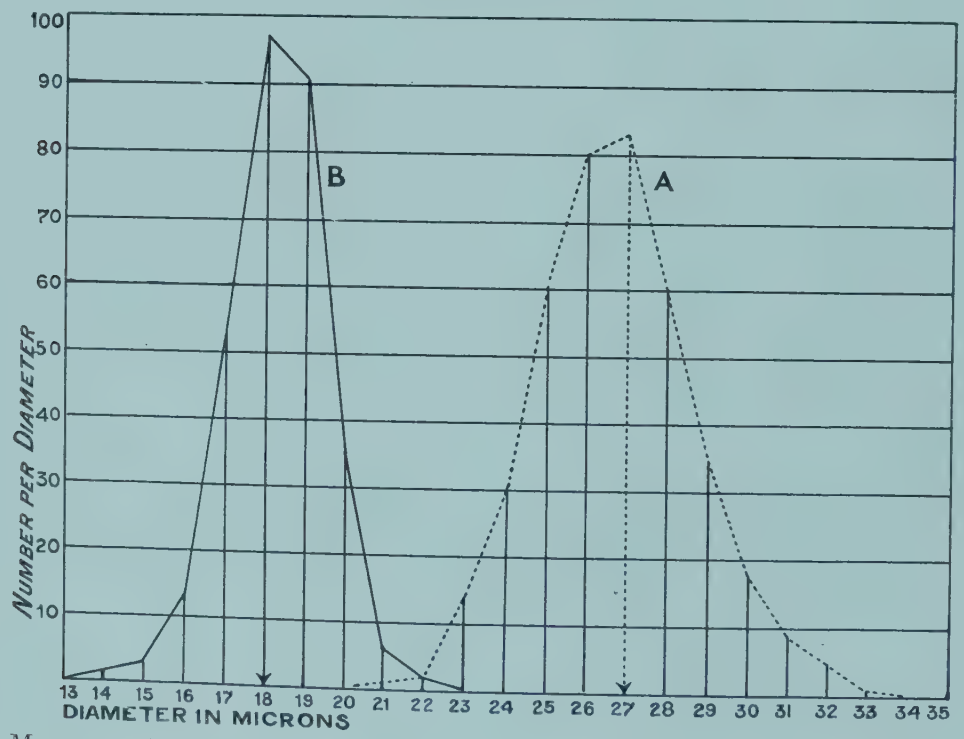


FIG. 2.—Measurements of oospores of *P. debaryanum* (300) and of *P. splendens* (400) plotted as frequency curve

(pl. 4, A). The most prolific appearance of these bodies was observed in colonies held at 30° C., which is also the optimum temperature for mass growth.

Germination in *A* may take place immediately after the conidium is cut in *Pythium complectens*. When placed in distilled or tap water, in potato juice, prune juice, and oatmeal decoction, from two to six tubes are put forth from each conidia, the number being larger in the richer substrata. The latter also induce more rapid and

abundant germination. Each germ tube becomes a "main" hypha and can be traced as such for a considerable distance; short side branches are given off profusely, with occasional longer lateral branches which soon acquire the greater thickness and length characteristic of the "main" hyphae.

Terminal microconidia, scarcely larger than the hyphal width, are sometimes found. The size of the normal conidia varies with the substratum, but is always much larger than that of *P. debaryanum* or *D* from the same medium. Culturing over a period of four years has not markedly affected the size of the conidia, although the oospores have become noticeably reduced in size.

Oospores of A.—Cultures of the original isolations were grown on various media for nearly two years before oospores were obtained. The first were found on a bit of old mycelium which had been used as inoculum for a plate of Knopf's synthetic agar, on which the organism makes a scanty, spreading growth. Subcultures of this, and inocula from two-months-old oatmeal agar cultures which had been kept in the icebox at least a month, subsequently yielded oospores quite regularly on Knopf's agar, later on cornmeal and carrot agars. The oospores appeared at first on and immediately around the inoculum; in later cultures they were produced in the general body of the medium, synchronously with the conidia. Oospores could usually be found in aggregates on distinct hyphal groups which could be traced some distance, indicating oospore production to be a function of special hyphae.

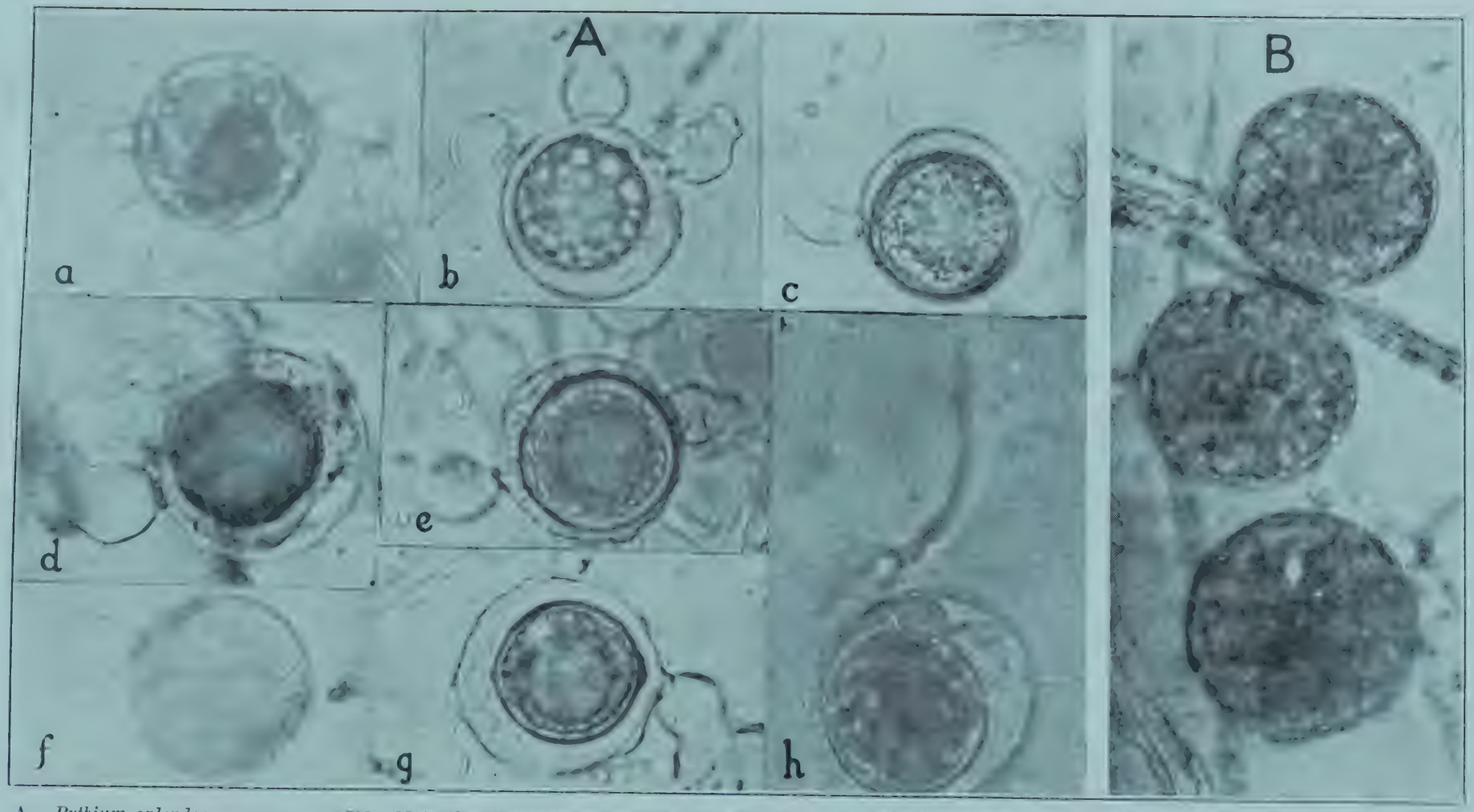
Heterothallism is, however, not indicated, since the cultures were descendants of single spore cultures which are readily made on account of the large size of the conidia. The factors responsible for sexual reproduction as contrasted with conidial reproduction are not clear-cut, since inoculations of a batch of plates occasionally resulted in purely conidial formation in some of the plates. Inoculations on cornmeal agar from old, chilled oatmeal agar cultures could generally be counted upon to produce oospores, though always in moderation.

The spherical oogonia of *A* are smaller than the conidia and contain a more finely granular, nonvacuolate hyaline protoplasm. They are soon enlaced by adjacent antheridia, which may arise below the oogonium or, more usually, from neighboring hyphae. They are clavate, much larger than those of *B* (compare pls. 6 and 5, which were photographed at the same enlargement), and from three to eight are found regularly with each oogonium. Each is fused at its apex with the oogonial wall and sends a fertilization tube across to the contracted oosphere. At maturity, all antheridia around the oospore are found empty. All do not, however, empty directly into the oosphere; observations on living material indicated that a membrane is formed after the emptying of one or possibly two antheridia into the oosphere, effectively preventing fertilization by the remainder. This is shown in Plate 6, A, *g*, where the contents of the left-hand antheridium have been extruded into the space between original wall and oosphere. The latter has evidently been fertilized by the antheridium at the right and formed a membrane before the others could empty into it, the membrane subsequently thickening into the oospore wall. Similar cases may be seen in Plate 6, A, *b* and *e*.

In one instance observed during fertilization a single antheridium of *A* had already emptied into the oosphere, which was rounded up and surrounded by a very thin membrane. A second antheridium was seen to extrude its contents through the fertilization tube which touched the oosphere membrane, the contents spreading out, however, over the oosphere surface and quickly retracting into the antheridium, where they degenerated into oily globules. Occasionally abortion takes place, such as was observed in *D*. The contracted oosphere fails to form a definite wall, turns into a mass of large, bright oily globules (pl. 6, fig. A, *b*), which expand and fill the oogonium (pl. 6, fig. A, *a*), later fusing into a single large vacuole nearly filling the oogonium except for a surrounding layer of granular, hyaline cytoplasm (pl. 6, A, *f*).

More frequently fertilization is followed by thickening of the oospore walls, accompanied by changes in the oospore contents, which result in the final appearance of a large excentric vacuole surrounded by a densely granular but hyaline cytoplasm. Both oogonial and oospore walls remain smooth and hyaline; the mature oospore lies free in the oogonium but occupies a space proportionally less than in the case of *B*. The ratio of oospore to oogonium in the former is 1 : 1.2, in the latter 1 : 1.1.

Germination of a few oospores of *A* was obtained by pouring aerated tap water over a month-old cornmeal agar plate culture containing oospores (pl.



A.—*Pythium splendens*, oospores. × 750. Note the numerous antheridia, the size compared with the oospores on Plate 5 (same magnification); germination shown in *h*

B.—Conidia of same. × 750. Compare with Plate 5, same magnification

6, A, *h*). The endospore wall is dissolved, and a single thick tube breaks the exospore and oogonial walls. The contents of the oospore gradually pass into the tube, which continues to grow and form a mycelium. Zoospore formation has not been observed.

CULTURAL STUDIES

The cultural characters of these fungi were studied along with those of *Pythium complectens*, on 16 media in 4 culture series. The terminology suggested by Harsch and Long (*13*) and also followed recently by Fritz (*11*) has been followed in describing the character of growth. These studies have shown specific differences which have remained constant since original isolation. Except on four media, the four fungi were readily distinguishable from each other macroscopically. Cultures of each organism were always made in triplicate tubes on each medium. Some of the more prominent diagnostic characters are as follows:

On oatmeal agar and on sugar-beet agar the growth of *A* plugged the tube up to the top of the slant, forming a narrow, compact web across the bore. This persisted when the rest of the mycelium below it had matted down. No such web was formed by the other fungi (pl. 7, A and E.)

The growth of *D* on most media was sharply characterized by being much more compact and appressed to the slant than that of *A* or *B*. This may be seen in the side views of the tubes in plates.

No aerial growth was produced by *A* on cornmeal agar, carrot agar, potato agar, potato dextrose agar, and potato cylinders (slight aerial on the last after four weeks), contrasting sharply with the abundant aerial growth of the other two fungi (pl. 8.)

A characteristic feature of *A* was the granular appearance of all cultures due to the abundant production of large conidia.

Growth of *B* on potato cylinders was distinctive, due to the abundance of loose, fluffy aerial mycelium. That of *A* was entirely prostrate, that of *D* was felty, compact and closely appressed to the cylinder.

The media used throughout was prepared uniformly in accordance with the formulas of the Laboratory of Plant Pathology. Directions as given by Smith (*19*) were followed in the case of nutrient beef-broth agar, potato cylinders, sugar-beet cylinders, and bean pods. The formulas for the remainder were taken from the files of the laboratory and are appended.

CORNMEAL AGAR

To 4 teaspoonfuls of corn meal add 1 liter of distilled water. Keep in water bath for 1 hour, temperature about 50° C. Filter through cotton. Add 1¼ per cent agar flour to the filtrate. Steam 1 hour, filter through cotton, tube, and sterilize in the autoclave for 15 minutes at 115° C.

OATMEAL AGAR

50 gm. oatmeal in 500 c. c. H_2O. Steam in bath; strain through gauze; add 2 per cent agar flour (mixed in cold water); make up to 500 c. c.; steam. tube, and autoclave 15 minutes at 115° C.

CARROT, SUGAR-BEET, STRING-BEAN AGARS

Wash thoroughly, weigh and add water to double the weight of solid material. Let simmer. Strain and add 1½ per cent agar flour; steam three-quarters of an hour, filter, tube, autoclave or steam. For geranium agar, use twenty times as much water, 3 per cent agar, and steam.

POTATO AGAR (STRONG INFUSION, OXIDIZED)

500 gm. potato, 1,000 c. c. distilled H_2O, 1½ per cent agar flour; weigh potatoes, put through meat grinder, add water, and let stand 1 hour. Strain through gauze to remove cellulose. Make up to volume. Steam juice to clarify, and filter through cotton. Add agar, steam three-quarters of an hour. Filter through cotton, tube, and autoclave for 15 minutes at 115° C.

Potato dextrose agar: Add 10 per cent dextrose.

CONGO-RED AGAR

To 1,000 c. c. distilled H_2O add 10 gm. saccharose (Mercks); 1 gm. K_2HPO_4 (dipotassium phosphate); 0.20 gm. $MgSO_4$ (magnesium sulphate); 15 gm. agar flour (powdered); 0.10 gm. congo-red (powdered Grubler's). Steam water and all salts for one-half hour, then add the congo-red. Filter through cotton and tube. Tube, autoclave for 15 minutes at 115° C.

MILK RICE (SOYKA)

(1) Measure out 50 c. c. nutrient bouillon and 150 c. c. milk and mix thoroughly. (2) Weigh out 100 gm. rice powder and rub it up in a mortar with the milk and broth mixture. (3) Fill the paste into sterile tubes. (4) Sterilize in the steamer at 100° C. for 30 minutes on each of 3 consecutive days. (A pure white opaque medium is thus formed.)

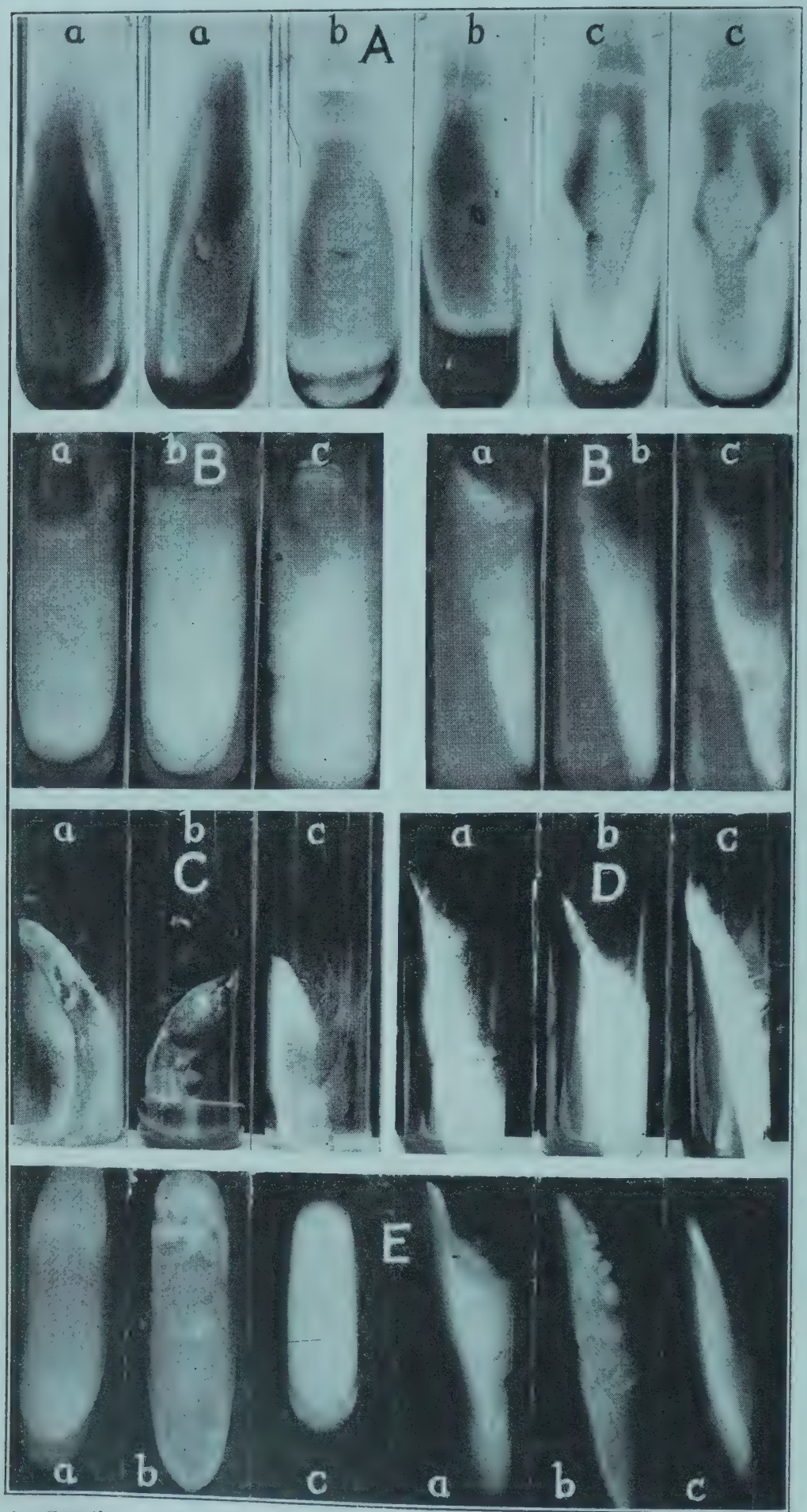
CORNMEAL FLASKS (FOR THE CULTIVATION OF FUNGI)

Into a 100 c. c. Erlenmeyer flask put a heaping teaspoonful of cornmeal. Put in enough distilled H_2O to thoroughly wet and with a little surplus. Stir round to mix, plug, and autoclave for 25 minutes at 120° C.

OATMEAL AGAR.—*A*. Abundant cobwebby white mycelium plugging tube up to top of slant; characterized by the formation of a compact web across the bore of the tube at the top of the slant, persisting after the growth below has matted down (three weeks). Vague outline at base.

B. Differs from *A* in the absence of a covering web, in the more compact mycelium, and in the sharp outline of the growth at the base of the slant against the glass.

D. More compact than *B*, white growth, plugging tube only halfway up slant; sharp outline at base.



A.—Growth on Congo Red agar; *a, a, Pythium splendens; b, b, P. debaryanum; c, c, P. debaryanum* var. *Pelargonii*
B.—Oatmeal agar; *a, a, P. splendens; b, b, P. debaryanum* var. *Pelargonii*. Front and side views
C.—Bean agar. Same lettering as in B
D.—Bean agar. Same lettering as in B
E.—Sugar-beet agar. Same lettering as in B

A.—Carrot agar

B.—Potato dextrose agar

SUGAR-BEET AGAR.—*A*. Growth similar to that on oatmeal agar, except that the mat at the base of the slant against the glass is thicker and sharply outlined; web present.

B. As in oatmeal agar, except for the indefinite outline at base.

D. As in oatmeal agar cultures, but more compact and appressed to slant, white at base, becoming deep olive buff at the tip where there is a persistent felty appressed mass.

BEAN AGAR.—*A*. First week's growth prostrate, forming a smooth thick mat, finely granular on account of the abundant large conidia; aerial growth during second week, cobwebby hyphae plugging lower part of slant up to third of slant, the rest more closely appressed, forming an open dry film; flattens down during third week to a sodden prostrate mat, except for a few small dry patches of aerial mycelium on the upper part of the slant.

B. Abundant aerial growth during the first week, plugging tube up to two-thirds of the way up the slant, more compact and felty at top of slant; drying down after three weeks to form an appressed thin film.

D. Aerial growth during first week, much more compact than *B*, flattening down to a thin dry film after three weeks.

POTATO CYLINDERS.—*A*. Prostrate, sodden wet mat over lower part of cylinder, scanty growth at top. Short downy white aerial growth after four weeks, matting down again in three months.

B. Abundant white aerial growth plugging part of the tube above slant within a week; becoming more compact in three weeks, entirely matted down in three months to a sodden glistening mass, with cobwebby strands from cylinder to glass.

D. Thick, white, closely appressed aerial growth within a week, more compact than *B*, not plugging tube, matting down in three months to a dry mat, dull. Smoke-gray to light grayish olive.

SOYKA RICE SLANT.—*A*. Compact, felty white growth, closely appressed to slant, later flattening down to a dry white film.

B. Abundant fluffy white growth plugging tube up to two-thirds of slant, flattening down after two weeks to a sodden mat.

D. Closely appressed aerial growth, more compact than *A*, forming a dry white film.

BEAN PODS.—*A*. Cobwebby film of mycelium covering pod and reaching to the glass, flattening down after two months to a sodden wet mat.

B. Tubes plugged up to top of pod with thick white mass of hyphae during first week; this mats down within 11 days to form a thick wet mat over the surface of the pod and water, leaving a few cobwebby strands bearing drops of exudate.

D. Early growth aerial, cottony, more compact than *B*, not plugging tube, remains aerial longer than *B*; mats down after three weeks.

SUGAR-BEET CYLINDERS.—*A*. Growth prostrate during first week, later aerial cobwebby to cottony in lower part, downy appressed at top; after three weeks flattening down to a sodden mat.

B. Abundant aerial growth from the start, plugging the tube up to top of slant with a thick weft of cottony white mycelium which flattens down after three weeks to a wrinkled wet mat with occasional hyphae between cylinder and glass.

D. Similar to *B*.

CARROT AGAR.—*A*. Growth prostrate, finely granular (due to conidia), smoke gray; small compact white tufts at top of slant; thin wefts of cobwebby aerial mycelium at edges of slant.

B. Abundant fluffy white growth, plugging tube four-fifths of the way up the slant; compact felty mass at tip; flattening down after three weeks, from base of slant upward to form a prostrate hyaline wet mat.

D. Differs from *B* in the very compact, appressed white aerial growth, not plugging tube; felty, closely textured at top, becoming cottony at the base; flattening down after a month to a thick dry mat.

POTATO DEXTROSE AGAR.—*A*. Similar to growth on carrot agar, except for the color, which is pale olive buff to light grayish olive; more pronounced aerial growth at edges of slant reaching to the glass.

B. Similar to growth on carrot agar.

D. Similar to growth on carrot agar, except that matting down takes place earlier in *D* than in *B* (two to three weeks), beginning at center and leaving cobwebby mycelium at the base and a smooth, closely appressed dry film at the tip.

POTATO AGAR, NO DEXTROSE.—*A*. Thick, prostrate, wet mat, uniformly granular, smoke gray to grayish olive, with slight felted aerial growth at upper half appearing after three weeks.

B. Cobwebby white aerial growth appearing during first four days, matting down within a week to form a prostrate gelatinous film, thickened at the base of the slant, pale smoke-gray to light grayish olive, aerial growth

again within three weeks, cottony at base, felted and compact at tip, matting down within three months.

D. The aerial growth is loose and more closely appressed to the slant.

CORNMEAL AGAR TUBES.—*A*. Thin, prostrate hyaline mat, thicker toward base of slant, finely granular due to abundant conidia. No aerial growth, except a dried felty layer at the tip.

B. Cobwebby white mycelium plugging tube up to half of slant, merging into a thin prostrate weft in the upper half; entire aerial mass flattens down to a gelatinous mat within two weeks.

D. Aerial growth less abundant than *B*, completely matting in 10 days.

CONGO-RED AGAR.—*A*. Scanty surface growth, except over lower half of slant, surface dry, shiny, abundant subsurface growth reaching throughout the agar, uniformly granular, conidia formed in abundance along the glass at the edge of the slant; prostrate except for cobwebby wefts at edges of slant appearing after two weeks. No color change after three months.

B. Abundant white surface growth, becoming aerial within a week, loose, cobwebby; plugging tube three-fourths up-slant; quickly matting down (two weeks) to a thin dry layer of closely appressed hyphae, aerial mycelium around edges persisting; abundant uniform subsurface growth of more compact texture than *A*. No color change after three months.

D. Differs from *B* in earlier and more abundant aerial growth, which is also much more compact and white and slower in matting down. No color change after three months. Note: Tubes of *P. complectens* showed a color change to Indian purple.

VIABILITY

Duplicate tube cultures of each organism on various media, which had been kept at room temperature (18° C. in winter to 35° C. in summer) were tested for viability as described for *Pythium complectens*. Melted cornmeal agar at 38° was poured over the slants after absorption of sterile oatmeal decoction; transfers from cultures which showed growth were compared with stock cultures and were also identified microscopically. The results are summarized below.

Isolation B.—Dead after 11½ months on potato agar, potato dextrose agar, Soyka rice, beef agar, geranium agar, and in one tube of oatmeal agar; also after 7 and 8 months on potato agar and Congo-red agar. Alive after 11½ months on cornmeal agar, bean agar, and in one tube of oatmeal agar; also after 5½ months on potato dextrose agar.

Isolation D.—Dead on all media kept longer than 5½ months.

Isolation A.—Dead on all media kept longer than 5½ months, except on carrot agar where it was recovered after 9 months.

The high viability of *B* as contrasted with that of the other two fungi, should be compared with the equally high viability of *Pythium complectens*, which was recovered after 11½ months on carrot agar and bean agar. There can be little doubt that the relative viability of these species of Pythium is correlated with the production of oospores, which is abundant in *P. debaryanum* and *P. complectens* and relatively scarce in the other two, as was pointed out in the description of their morphology.

TEMPERATURE RELATIONS

The accompanying chart (fig. 3) outlines the growth curves averaged from two experiments with plates in ice thermostats and warm incubators, ranging from 3° C. to 35.5°. Triplicate plates of cornmeal agar of each organism were placed in each compartment and measurements of the colony diameter were made at 24-hour intervals. Inoculations were made from a three-days old cornmeal agar plate culture which was cut up into one-sixteenth inch squares, each square then being planted in the center of the fresh plate. Before inoculation all plates were kept overnight in their respective compartments to avoid lag effects.

The behavior of these fungi at the lower temperatures presents some interesting features.

Isolation *D* shows the lowest minimum, the colonies growing appreciably at 6° C. within 24 hours, and reaching a diameter of 41 mm. in 144 hours (not shown on chart). Growth was evident at 3° within 48 hours, and, when placed directly on ice, showed a slow growth at 96 hours, a characteristic separating it immediately from the remaining Pythium species studied. In fact, the quickest way of differentiating this organism from the others was to place freshly inoculated plates in the lowest-temperature compartments; the plate showing the most growth, or in fact any growth at all at or near 6°, within 24 hours, could be counted on as being isolation *D*.

Colonies of isolation *B* showed growth at 6° only after 72 hours, reach-

ing 7 mm. at 144 hours; no growth was observed at 3°.

Isolation *A* showed the highest minimum, showing no growth at 6° even after 144 hours and at 11° only after 72 hours.

The optimum temperatures of the three forms do not differ greatly, best growth taking place between 27° and 30°. Between 20° and 35.5°, however, the growth of *A* constantly outstripped that of the other two fungi. It was also characterized by a higher maximum.

The temperature experiments indicate distinct physiological differences between these fungi, which are particularly evident and constant in the ability to grow at low temperatures, and in the greater luxuriance of growth of *A* within the range of temperatures at which cultures are usually incubated.

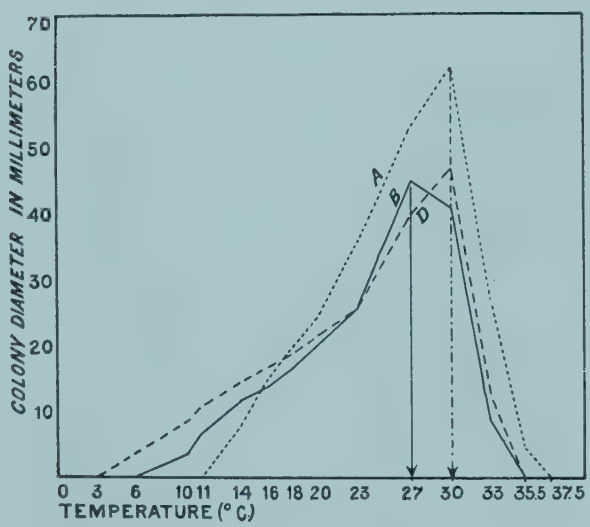


FIG. 3.—Temperature relations plotted in colony diameter growth for 25 hours

TAXONOMY

The morphological character of these fungi places them in Butler's group of Pythium species which includes forms with a smooth-walled oospore lying free in a smooth-walled oogonium, and with spherical conidia or sporangia. These include (1) *P. debaryanum*, (2) *P. vexans*, (3) *P. ultimum*, (4) *P. anguillulae aceti*. The first is considered identical with the writer's isolation *B* in measurements, relative abundance of oospores and conidia, in cultural characters so far as detailed by Hawkins (*14*) and by Edson (*10*), and in appearance as figured by De Bary, Ward, and others. Zoospores were not obtained, but neither were they found by Ward, Edson, or Johnson (*16*). It also corresponds with *P. debaryanum* as figured from geranium by Peters (*17*), although the originality of his drawings is not clear in view of the fact that he repeated Hesse's mistake of placing a single cilium on the zoospores, the biciliate nature of which was pointed out by De Bary (*3*).

Isolation *D*, while corresponding closely to *P. debaryanum* in measurements, differs in the following respects: (1) Constant and readily distinguishable cultural differences; (2) specific temperature relations, evident by growth at the lowest temperature of the four species of Pythium studied; (3) a distinctly slower infection rate as compared with *B* and *A;* (4) a preponderant tendency toward a sexual reproduction, oospores appearing only rarely and then often aborted; (5) a thinner oospore wall; (6) greater variation in the size of conidia as compared with *B;* (7) the appearance, within a short time after inoculating plates, of irregular swollen bodies formed by the retraction of protoplasm with the laying down of successive septa, germinating by a germ tube and also differing in size and absence of lobing from the "presporangia" of *P. butleri.*

In the above respects *D* does not correspond with any of the other species grouped with but distinguished from *Pythium debaryanum.* In its parasitism and scarcity of oospores it differs from *P. ultimum; P. intermedium,* in which oospores are unknown, is characterized by catenulate conidia, which are not present in this fungus. The writer considers it a variety of *P. debaryanum,* to which it is clearly closely related, and proposes the name *Pythium debaryanum* var. *pelargonii.* The technical description follows:

Pythium debaryanum var. **pelargonii** nov. var.

Hyphae coenocytic, hyaline, richly branching from thick main hyphae, 3.8 μ to 9.8 μ wide, average 6.2 μ; oospores spherical or subspherical, smooth, diameter 15.9 μ to 19.9 μ, average 17.8 μ; free in the smooth-walled oogonium, thin-walled, scarce in culture media, often aborted; oogonial diameter 17.4 μ to 21.9 μ, average 20.1 μ; one to four antheridia, clavate, often adhering to the oogonium along their entire length, usually arising from a neighboring branch; conidia spherical or subspherical, terminal and intercalary, smooth, diameter 12.8 μ to 27.7 μ, average 20.1 μ; hyaline, germinating by one to three tubes, formed abundantly on media; sickle-shaped bodies formed singly or in chains two days after inoculations of plates; irregular swollen intercalary bodies formed in plates within seven days after inoculation, by

retraction of protoplasm with laying down of successive septa, germinating by germ tube; minimum temperature 6° C. at 24 hours, lower than *Pythium debaryanum*, from which it is further distinguishable culturally, particularly in a more compact habit of growth. Parasitic on Pelargonium sp., causing a stem rot.

In the case of *A*, the smooth oospore lying free in the smooth oogonium (specific according to Butler) separates it immediately from other large-conidial and multi-antheridial species. Conidia are unknown in *Pythium proliferum*, whose zoosporangia are large, spherical, and proliferating, with oospores not filling the oogonium. *P. megalacanthum* is multi-antheridial, but the oogonial walls are sculptured.

Type *A* differs from *Pythium debaryanum* and the allied species both morphologically and physiologically, in the following respects: (1) The constant larger size of the fruiting bodies compared with those of the other fungi studied on the same media; (2) constant and recognizable cultural differences; (3) preponderance of conidial production; (4) specific temperature relations, expressed in a higher minimum, and in more luxuriant growth than the others at 20° to 35.5° C.; (5) difference in the size ratio of oospore to oogonium; (6) constant accompaniment of the oogonium by more than one antheridium, ranging from three to eight under conditions identical with those at which *P. debaryanum* produced one or rarely more than two; it differs from *P. ultimum* in parasitism, measurements and absence of intercalary conidia, and from *P. intermedium* in the absence of catenulate conidia. It is considered a distinct species, for which the name *Pythium splendens*, n. sp., is proposed. The technical description is appended.

Pythium splendens, n. sp.:

Hyphae 3.5 to 9.2 μ wide, average 6.4 μ; oospores spherical to ellipsoid, smooth walled, lying free in the smooth oogonium; ratio oospore to oogonium 1:1.2; oospores thick walled, produced sporadically in cultures; three to eight antheridia, clavate, arising at the oogonial stalk and from neighboring branches; oospores germinating by germ tube within a month of formation; conidia spherical, always terminal, smooth walled, large, 21.7 μ to 48.9 μ in diameter, average 36.2 μ; hyaline, darker on rich media, germinating by two to six germ tubes; sickle-shaped, clavate bodies formed singly and in chains on plate media two days after inoculation; temperature ranges 10° to 37.5° C., optimum 30°; minimum higher than that of *P. debaryanum* and more luxuriant growth between 20° and 35.5; readily distinguishable by specific cultural characters. Parasitic on Pelargonium, in the tissues of which conidia are abundantly formed.

SUMMARY

Stem rots of Pelargonium cuttings caused by *Pythium debaryanum*, *P. debaryanum* var. *Pelargonii*, nov. var., and *P. splendens*, n. sp., are described and the comparative morphology and physiology of these organisms is detailed.

The signs of the disease consist of a blackening and shriveling, starting at the cut base and ultimately involving the entire plant in a soft rot. *P. debaryanum* var. *Pelargonii* differs, so far as infection is concerned, only in the slower progress of the discoloration.

Inoculation experiments with single-spore cultures and subsequent reisolations have demonstrated the pathogenicity of these organisms on Pelargonium, coleus, begonia, cucumber seedlings, and radish seedlings, *P. debaryanum* proving the most pathogenic on the last two.

Cultural characters on 16 media are described. The three fungi are readily distinguishable macroscopically.

Pythium debaryanum var. *Pelargonii* is characterized by the lowest minimum temperature, a character sufficiently constant and specific to identify it experimentally within 24 hours. *P. splendens* shows the most luxuriant growth of the three at 20° to 35.5° C.

Pythium debaryanum shows the highest viability on media and was readily recovered after 11½ months at room temperature. The other two were dead after 5½ months, except *P. splendens*, which was recovered in one case after 8 months. The correlation between oospore production and viability is pointed out.

Pythium debaryanum var. *Pelargonii* is characterized morphologically by greater size variation of conidia and their preponderance over oospores, which are produced only rarely and are often aborted; its oospores are thin-walled; one to four antheridia are present; irregular swollen intercalary bodies are formed through retraction of the protoplasm, which lays down successive septa; these resting bodies are not related to aging of the media but are produced within seven days of plate inoculation.

Pythium splendens is characterized morphologically by larger conidia, oospores, and oogonia; by the constant presence of three to eight antheridia; by the terminal formation of conidia, no intercalary ones being found; by the preponderance of conidia, oospores being formed only sporadically; by germination of conidia with two to six germ tubes.

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VARIATION IN THE KHERSON OAT AT AKRON, COLORADO [1]

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INTRODUCTION

Few oat varieties grown in the United States surpass the Kherson in economic importance or in potential value. It is one of the most widely distributed early varieties, especially in the Corn Belt and the central section of the Great Plains area. The extensive distribution of Kherson and Sixty-Day oats in the United States in 1919 is shown by the map (fig. 1) prepared from statistics obtained in an oat varietal survey conducted by the Office of Cereal Investigations in cooperation with the then Bureau of Crop Estimates. Among the 10 leading varieties in 1919, Kherson and Sixty-Day ranked fourth in acreage. When the acreages of important selections such as Albion (Iowa No. 103), Iowar, Gopher, Richland (Iowa No. 105), and States Pride (Wisconsin No. 7) are added to those of the original variety, this type of oat easily ranks third in importance in the United States, being exceeded only by Silvermine and Red Rustproof.

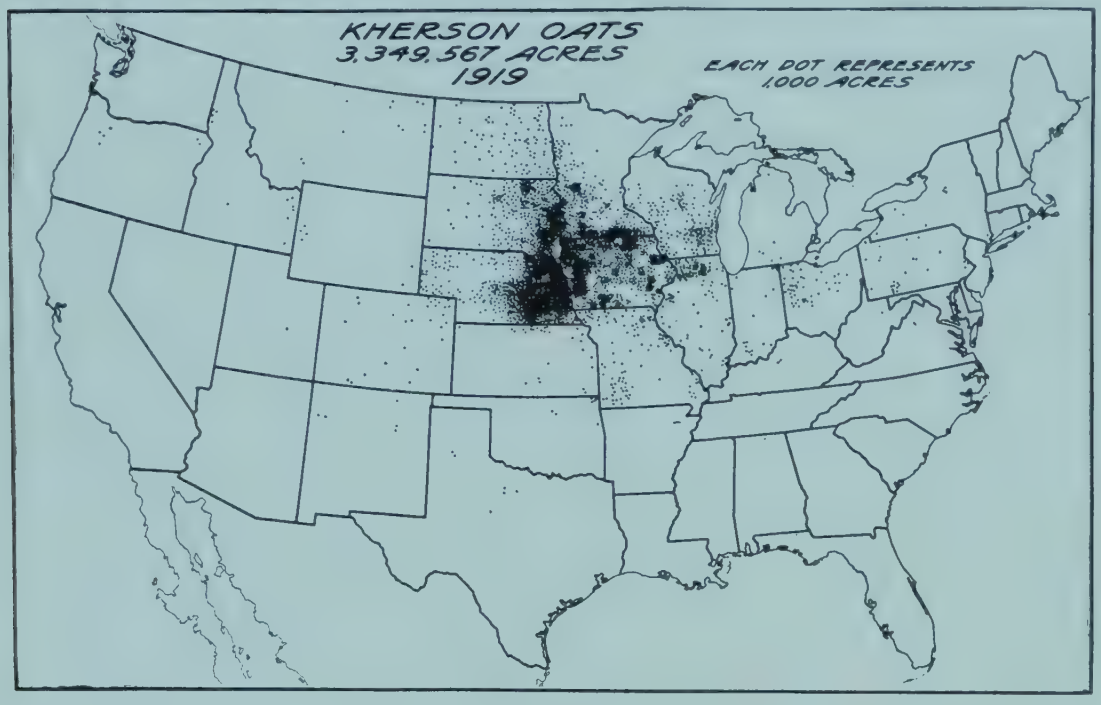

FIG. 1.—Outline map of the United States, showing the distribution of Kherson (Sixty-Day) oat in 1919

REVIEW OF LITERATURE

Carleton (*2*, *3*)[3], Lyon (*15*), Warburton (*24*), and Warburton and Stanton (*26*) have presented historical accounts of the introduction of the Kherson and Sixty-Day into the United States. All of the foregoing writers, and also Etheridge (*5*), have classified Kherson as belonging to *Avena sativa* and have published general or botanical descriptions of the variety. Warburton and Stanton (*26*) state that "botanically the Kherson and Sixty-Day oats can not be distinguished one from the other, * * * the varieties are

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[3] Reference is made by number (italic) to "Literature cited," p. 1081.

identical and the names, therefore, are synonymous * * *." They are so considered in this paper.

Norton (*18*) points out that most oat varieties probably consist of numerous strains. The work of Coffman, Parker, and Quisenberry (*4*) shows this to be especially true of the Burt variety, which was found to consist of numerous distinct types, some of which differ widely. Warburton and Stanton (*26*) have stated that both white-kerneled and yellow-kerneled selections have been made from Kherson or Sixty-Day. Stanton (*19*) and many others have made reports on experiments conducted with selections from this variety. Reports by Love (*10, 11, 12*), Williams and Welton (*28*), Warburton, Burnett, and Love (*25*), Surface and Zinn (*21*), Welton and Gearhart (*27*), Kiesselbach and Ratcliff (*8*), Burnett (*1*), Hayes and Garber (*7*), Leith and Delwiche (*9*), and Burnett, Stanton, and Warburton[4] have described selections made from Kherson or Sixty-Day.

Panicles and spikelets of important selections from Kherson or Sixty-Day are shown in Plate 1.

Many investigators have used strains of Kherson or Sixty-Day as parental material in oat hybridization, both with the object of producing improved economic varieties and for genetic studies. A correct knowledge of the variety itself is therefore of considerable importance.

Surface (*20*) made one of the first reports on a cross in which the Kherson oat was studied genetically. He crossed this variety on *Avena fatua*, the common wild oat. He states that the pure-line selection of Kherson used in this cross had been grown for five years and had always bred true for all characters. According to him the awn was seldom present in this strain of Kherson. When present it was found only on the lower kernel and then was very weak. The kernels of this selection had no basal scar and seldom had basal hairs, although spikelets having lower kernels with one or two long hairs on the side of the callus occasionally were found. He further states that all cultivated varieties of oats which have come to his notice may have this slight pubescence at the base of the lower grain and that some varieties have it more marked than others. Surface also found the F_1 of his cross to be intermediate between the two parents in nearly all respects. In F_2 he observed that the basal scar segregated into a ratio of one prominent, two intermediate, and one absent. He observed linkage between basal scar and the presence of hairs on the base. The absence of basal scar was found to be apparently dominant, or at least partially so, over the "wild" type of base having a very pronounced scar. No marked relation was noted between the yellow color of the Kherson strain he used and absence of awns. He attributed the fact that F_2 awnless plants failed to breed true in F_3 either to the presence of additional genes or to the explanation of Nilsson-Ehle (*17*) that external conditions may greatly affect the production of awns in cultivated oats. Surface further states: "General observations also indicate that the character of awning varies widely even within a pure line, and it may happen that plants which are genetically awned will, because of environmental or other conditions, show no awns." He found evidence of linkage between cultivated base form and absence of hairs and also between yellow color and absence of basal scar.

Love and Fraser (*13*) crossed awnless Sixty-Day with Burt and with Red Rustproof (Red Texas), both of which bore awns of the "weak" type. The F_1 of both crosses was almost awnless. They observed that awnless F_2 plants did not always produce progeny all of which were awnless in F_3, a fact which they attribute to environmental factors preventing or obscuring the production of awns in the F_2 generation. They believe the "strong" awn type to be recessive, but the awnless condition could not be considered as being entirely dominant. Apparently the explanation of Nilsson-Ehle (*17*), which assumes that yellow color in oats is linked with an inhibitory factor for awn production, is favored. It is suggested by Love and Fraser that probably the results obtained by Surface (*20*) in crossing Kherson oats with the wild *Avena fatua* may be similarly explained—i. e., Kherson may carry a factor inhibitory to awning.

Love and Craig (*14*) report results obtained in crossing *Avena fatua* × *Avena sativa* variety Sixty-Day. Their results, however, do not agree with those of Surface. They point out that in view of the possibility of obtaining different strains from a variety, particularly so far as the inheritance is concerned, as shown by yield, etc., it is not surprising that these results should not agree. Love and Craig conclude that the Sixty-Day strain they used

[4] BURNETT, L. C., STANTON, T. R., and WARBURTON, C. W. IMPROVED OAT VARIETIES FOR THE CORN BELT U. S. Dept. Agr. Bul. 1343. 1925. (In press.)

Panicles and spikelets of Kherson and some yellow and white kerneled selections

A. (1) Kherson; (2) Richland (Iowa No. 105); and (3) States Pride (Wisconsin No. 7)
B. (1) Albion (Iowa No. 103); (2) Iowar; (3) Nebraska No. 21; and (4) Cole

carries an inhibitor for awning which is linked with yellow color, and state that the third generation tends to substantiate the conclusions drawn from the study of the second generation. They also observed in F_2 and F_3 correlation between yellow color and the absence of basal scar as well as between yellow color and absence of basal hairs.

Fraser (*6*) discusses the results obtained in the study of crosses, Sixty-Day × Burt and Sixty-Day × Early Ripe (Burt). He believes that the Sixty-Day carries the factor for awning, but that it is prevented from operating in the cross by an inhibitor which is closely linked with the factor for yellow color in the Sixty-Day variety. He attributed the production of awns in the first generation to the extent to which this inhibitor (I), is dominant over its normal allelomorph (i), which in turn is probably dependent to a large extent on environmental factors. He believes that environment influences the production of awns and states that, though experimental evidence is lacking, increased moisture and fertility of the soil tend to decrease their number. According to Fraser, the variety Sixty-Day would have the genetic formula for color rryyYY as contrasted with the formula RRYYyy of the variety Burt. He states further:

> Other workers have shown that the variety Sixty-Day carries with it a factor which inhibits the production of awns, which factor is closely linked with the factor for yellow color. Because of the yellow in the variety Burt, which carries no inhibitor, the inhibitory effect of the Sixty-Day factor was obscured.

Fraser says that considerable variation in kernel color is to be noticed even within the same pure line during different seasons or under strikingly different environments. He speaks of Sixty-Day as being a yellow variety and found white F_2 plants which failed to breed true in F_3. The difficulty of making exact color classification due to gradation is also pointed out. Linkage was observed between the fully awned condition, the presence of midlength basal hairs, and the Burt (sterilis) type of articulation.

CHARACTERS OF THE KHERSON SPIKELET

A brief description of the oat spikelet is given to make clear the discussion of the experiments which follow. The principal spikelet characters studied, as in previous similar investigations by Coffman, Parker, and Quisenberry (*4*), were spikelet disarticulation, floret disjunction, basal hairs, awns, and lemma color.[5]

The oat spikelet is borne on the end of the pedicel, terminating in the lower segment of the rachilla. Each spikelet contains two or more florets, of which usually only the two lowest are fertile, the lower one of the two being the larger and longer. The outer or empty glumes are thin, membranous, broadly lanceolate, pointed, glabrous, and broadly arched. The upper is a little longer than the lower and both exceed the lemma or flowering glume in length, except in the hull-less or naked group. There are no varieties bearing exclusively one, two, or three kernels per spikelet. Two or more usually occur and may or may not be separated in threshing. The florets are connected by the clavate segments of the jointed rachilla, each segment of which supports a single floret.

SPIKELET DISARTICULATION

The separation of the spikelet from the plant by disarticulation at the juncture of the lower floret and its supporting rachilla segment has been fully discussed in the previous paper on the Burt oat (*4*). In the present study only two distinct forms of lemma base resulting from spikelet disarticulation were recognized. The oval smooth-edged and rather prominent cavity or scar resulting from abscission, usually found in oat kernels of the Red Rustproof type, was not observed in this study of Kherson. In the present study, therefore, spikelet disarticulation was classed as by semiabscission and by fracture. The pointed form of base resulting from fracture is commonly associated with oats of the *Avena sativa* group. The term semiabscission was used for those kernels which showed a slight or poorly developed basal cavity resulting partly from abscission and partly from fracture.

FLORET DISJUNCTION

The manner of separation of the kernels of the spikelet varies with the species. In some species, as in *Avena fatua* and its derivatives, disjunction of the upper floret from its supporting rachilla segment takes place in approximately the same manner as does that of the spikelet. In *Avena sterilis* and its derivatives the rachilla and the lemma of the upper kernel are solidly grown together; the kernels do not

[5] For assistance in determining the morphologic characters of the oat spikelet and for the terminology used the writers are greatly indebted to C. R. Ball, senior agronomist in charge of Cereal Investigations.

separate readily in threshing, but the rachilla segment tears away at or near its base. At other times disjunction may result by the connecting segment splitting lengthwise or breaking at or near the mid-point.

Floret disjunction in this study was described as resulting by disarticulation when the separation left the rachilla segment attached to the face of the lower floret; by heterofracture when the rachilla segment broke at or near the mid-point, making exact classification in either of the other classes impossible; and by basifracture when the rachilla broke off at the base and remained attached to the second floret.

BASAL HAIRS

Most species of wild oats are characterized by hairiness of the lemma, callus, and rachilla. The callus often bears more or less conspicuous hairs or bristles, usually conveniently called basal hairs. Their presence may be observed readily without magnification. These hairs vary in number and length. Different authors have classified them in different ways, but all have used the length or number or combinations of these in making their classifications.

In the Kherson variety all hairs are short, with but few exceptions. On an occasional individual they might be termed midlength, but none observed were of sufficient length to be classed as long. As a result classification of basal hairs in the present study is based almost entirely on numbers of hairs. The occasional kernel with midlength hairs was arbitrarily thrown into the class with short hairs. All gradations from the very shortest hairs visible to the eye up to those of midlength were observed. In this study of Kherson the basal hairs were described as abundant, few, and absent.

AWNS

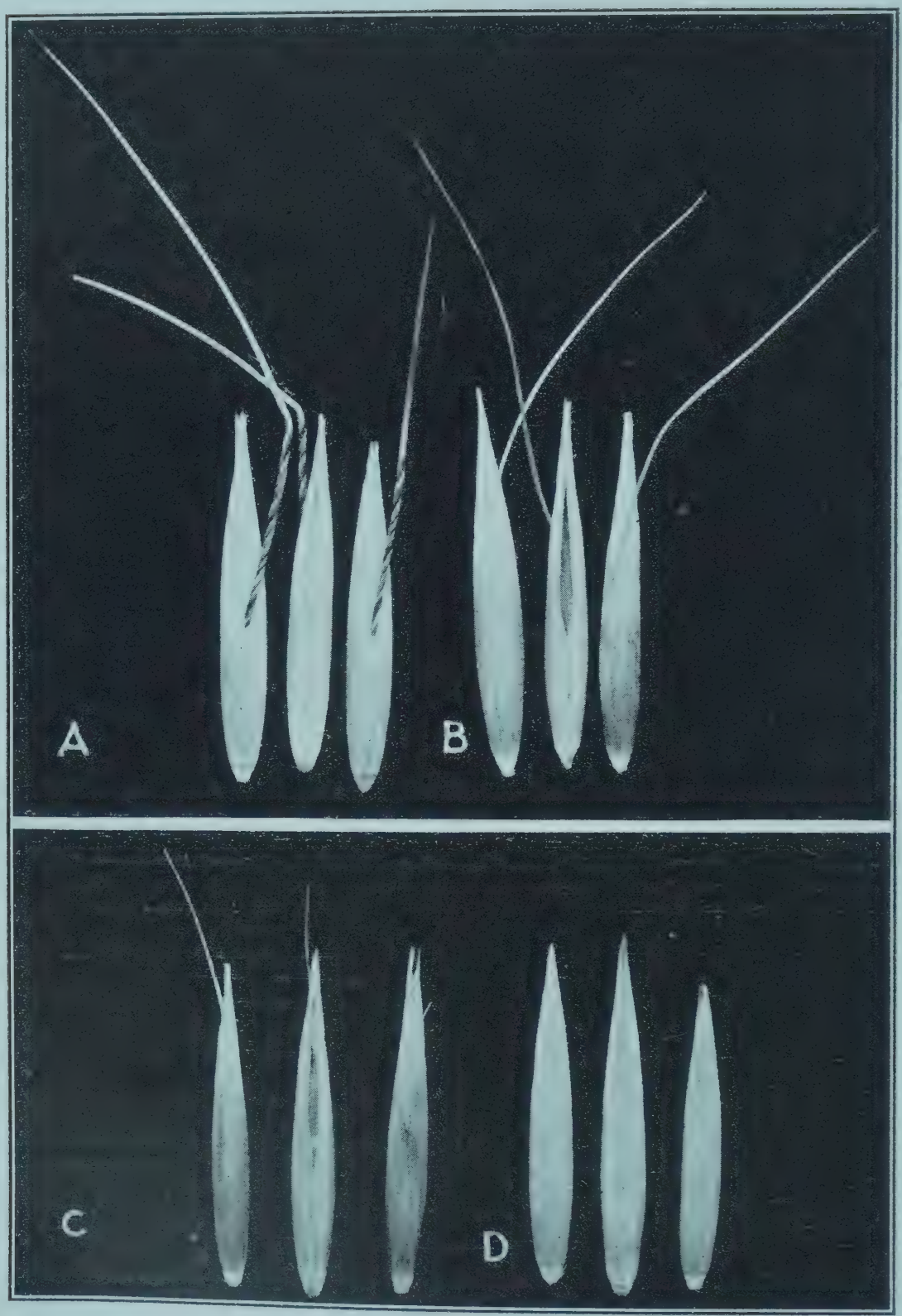
In Avena the awn is an extension of the midrib of the lemma arising from the epidermis at a point usually slightly above the middle of the dorsal surface of the kernel. In the various wild forms awns also occur on the second and third kernels. In such forms the awn usually is stout and long and the basal portion strongly twisted in a clockwise direction. The upper portion usually is bent or geniculate. In most of our cultivated varieties the awn occurs on only the lower kernel of the spikelet, which may be twisted or nontwisted and straight. In practically all the varieties of *Avena sativa* the awn occurs only on the lower lemma, and there are some varieties in which awns occur only occasionally or rarely even on the lower floret. In the varieties of *A. byzantina* awns often occur on both florets, and in the less variable varieties such as Red Rustproof and Red Algerian the awn usually is straight and rarely twisted. Trabut (*22*, *23*) observed a series of oat forms ranging from the wild red oat, *A. sterilis*, to the cultivated red oat which he called *A. sterilis culta*, and noted gradual reduction in the number of awns per spikelet and in the amount of twisting and geniculation. The occurrence of numerous twisted geniculate awns in cultivated oats is believed by some to indicate degeneracy resulting primarily from an unfavorable environment.

Various authors have used different terms to describe the nature of the awn. Etheridge (*5*) used the following terms: Twisted geniculate, strong, and weak. Fraser (*6*) in his studies of the inheritance of what he called the weak awn in certain oat crosses used the terms strong, intermediate, and weak to designate different awn types.

The following terms, indicating four classes, have been used in the present investigations: Twisted; nontwisted, long; nontwisted, short; and absent (awnless) (pl. 2). While the twisted geniculate awn usually is associated with wild forms, some cultivated varieties, such as Swedish Select, frequently show this type of awn to a marked degree. In the present study all awns showing some twisting were classed as twisted, regardless of the degree of geniculation. The nontwisted long awns often were as long as the twisted awns but were never twisted. The nontwisted short awns varied from approximately 15 mm. in length to mere bristlelike appendages.

LEMMA COLOR

In oat varieties the lemma varies in color. The principal colors recognized in descriptions of oat lemmas are black, red, gray, yellow, and white. Lemma colors in the Kherson variety were described in this study as reddish yellow or orange, yellow, and white. Lemmas called reddish yellow are of a dark orange or reddish color. The yellow class included all lemmas showing yellow color and varied from a rich cream to dark lemon-yellow. Lemmas classed as white were of an ivory or light cream color. Pure white lemmas probably do not exist in oats.



Awn types of Kherson oat

A. Twisted
B. Nontwisted long
C. Nontwisted short
D. Absent (awnless)

EXPERIMENTAL METHODS

The review of selection and other experiments has shown conclusively that the Kherson variety contains different types. The experiments described in this study were started at the Akron Field Station in 1921 to obtain information on the genetic constitution of Kherson, particularly with regard to certain spikelet and floret characters.

Kernels used in starting this study were selected singly from a bulk sample of Kherson oat, C. I. No. 459,[6] grown at the Akron Field Station in 1920. The kernels were classified and described by the system outlined by Coffman, Parker, and Quisenberry (*4*) in studies of the Burt oat.

The same general system in choosing seed and in planting was used each year. In all cases the crop was grown in the screened breeding garden at the Akron Field Station. In making the original seedings, kernels having an identical classification were sown together as a group. About 125 kernels were sown in 1921. The kernels were spaced at 3-inch intervals in rows 10 inches apart. The seed was sown on May 12 and the seedlings emerged May 21. The date of heading of each plant was recorded on a tag attached to the plant. Some plants did not mature seed because of unfavorable weather conditions.

The different plants showed considerable variation in time of heading and ripening. The earliest plants started to head July 2, while the latest date of heading was July 20. Most of the plants headed between July 10 and 16. At harvest the plants were pulled, and those of each group were tied together and stored. Later, each plant of the group was numbered, and the height of culms, number of culms, number of panicles, length of main panicle, and date of heading were recorded.

The primary floret in the spikelets, rather than the plant, was used as the unit throughout this study. In 1921 each of the primary florets from the main panicle of each plant was described. In succeeding years 25 florets per plant were described, and where the main panicle contained less than 25 spikelets more than one panicle on the same plant was used in order to obtain a sufficient number for accurate classification. The characters recorded were the same as those used in describing the original seed.

All of the distinct characters and, so far as practicable, all of the different combinations of these characters were included in the 1922 studies. In making the selections for seeding in 1922 there were sown not less than 5 nor more than 10 kernels having the same classification and from any one plant. In a few cases two groups of five kernels each from a single parent plant were sown. In such cases these groups differed in one or more characters, e. g., group 34 and group 35 both came from plant 8 of the 1921 crop (plant 8, group 14). Kernels in group 34 bore long awns in 1921, while those in group 35 were awnless.

About 250 kernels were sown in the 1922 experiments. The seed was sown April 22 and the plants emerged May 5. An excellent stand was obtained and the plants made rapid growth. As the season was dry, it was necessary to irrigate them several times.

In 1923 20 groups of kernels were sown. The seed was sown on May 7 and the plants emerged May 15. Excellent stands were obtained and the plants in most rows made very satisfactory growth.

EXPERIMENTAL RESULTS

About 7,000 kernels were described in the course of this experiment, and it is impracticable to include more than a general summary of the data obtained. In discussing the results the characters studied have been considered separately.

SPIKELET DISARTICULATION

In these experiments no florets were found which had a prominent basal cavity or scar resulting from disarticulation by abscission, such as is characteristic of *Avena sterilis*, *A. fatua*, and some varieties of *A. byzantina*. Should kernels of this type occur in the Kherson oat, it might well be considered evidence of mechanical mixture or of hybridization. Only two methods of spikelet disarticulation were observed in Kherson. These were semiabscission, resulting in a slight or indistinct cavity, and fracture, resulting in a rough surface with no visible scar. The data obtained in 1921, 1922, and 1923 on the inheritance of spikelet disarticulation are shown in Table I. It appears evident that heritable variations exist in the method of spikelet disarticulation of different pedigreed strains of Kherson oat. Apparently a strong tendency exists for disarticulation by semiabscission and by fracture to be transmitted to the progeny in a high proportion of cases.

[6] Accession number of the Office of Cereal Investigations.

TABLE I.—*Inheritance of spikelet disarticulation in strains of the Kherson oat grown at the Akron Field Station, Akron, Colo., in 1921, 1922, and 1923*

DATA FOR 1921

Spikelet disarticulation of parent and group number	Spikelet disarticulation in progeny			
	Number of kernels disarticulating by—		Percentage of kernels disarticulating by—	
	Semiabscission	Fracture	Semiabscission	Fracture
Semiabscission:				
4	1	18	5.3	94.7
5	124	13	90.5	9.5
12	55	55	50.0	50.0
All groups	180	86	67.7	32.3
Fracture:				
1	------	86	------	100.0
2	25	53	32.1	67.9
3	5	8	38.5	61.5
6	23	40	36.5	63.5
7	31	107	22.5	77.5
8	42	28	60.0	40.0
9	------	71	------	100.0
10	------	37	------	100.0
11	------	42	------	100.0
13	8	54	12.9	87.1
14	112	78	58.9	41.1
15	34	18	65.4	34.6
All groups	280	622	31.0	69.0

DATA FOR 1922

Semiabscission:				
6	38	36	51.4	48.6
7	17	55	23.6	76.4
8	50	107	31.8	68.2
9	84	1	98.8	1.2
10	35	34	50.7	49.3
11	23	57	28.8	71.2
14	20	10	66.7	33.3
15	25	72	25.8	74.2
22	80	20	80.0	20.0
23	52	12	81.2	18.8
24	68	3	95.8	4.2
32	78	2	97.5	2.5
33	58	17	77.3	22.7
34	119	5	96.0	4.0
35	58	------	100.0	------
37	49	32	60.5	39.5
40	97	33	74.6	25.4
41	66	9	88.0	12.0
All groups	1,017	505	66.8	33.2
Fracture:				
1	------	223	------	100.0
2	------	105	------	100.0
3	------	64	------	100.0
4	4	80	4.8	95.2

TABLE I.—*Inheritance of spikelet disarticulation in strains of the Kherson oat grown at the Akron Field Station, Akron, Colo., in 1921, 1922, and 1923*—Continued

DATA FOR 1922—Continued

Spikelet disarticulation of parent and group number	Spikelet disarticulation in progeny			
	Number of kernels disarticulating by—		Percentage of kernels disarticulating by—	
	Semiabscission	Fracture	Semiabscission	Fracture
Fracture—Continued.				
5	61	44	58.1	41.9
12	3	97	3.0	97.0
13	73	122	37.4	62.6
16	------	26	------	100.0
17	66	50	56.9	43.1
18	5	108	4.4	95.6
19	3	64	4.5	95.5
20	29	57	33.7	66.3
25	34	66	34.0	66.0
26	40	38	51.3	48.7
27	------	56	------	100.0
28	------	127	------	100.0
29	16	57	21.9	78.1
30	16	59	21.3	78.7
31	14	102	12.1	87.9
38	------	30	------	100.0
39	------	17	------	100.0
All groups	364	1,592	18.6	81.4

DATA FOR 1923

Semiabscission:				
4	2	123	1.6	98.4
5	93	4	95.9	4.1
10	80	45	64.0	36.0
11	118	7	94.4	5.6
15	37	15	71.2	28.8
16	75	4	94.9	5.1
18	112	------	100.0	------
19	100	------	100.0	------
20	78	------	100.0	------
All groups	695	198	77.8	22.2
Fracture:				
1	------	93	------	100.0
2	91	34	72.8	27.2
3	36	66	35.3	64.7
6	18	51	26.1	73.9
7	------	52	------	100.0
8	------	136	------	100.0
9	5	133	3.6	96.4
12	------	109	------	100.0
13	------	85	------	100.0
14	2	41	4.7	95.3
17	------	125	------	100.0
All groups	152	925	14.1	85.9

In 1921 parental florets disarticulating by semiabscission produced progeny of which two-thirds disarticulated by semiabscission and one-third by fracture. Parental florets disarticulating by fracture produced progenies in 1921 of which one-third disarticulated by semiabscission and two-thirds by fracture. The results obtained in 1921 indicate that a definite relation exists between parent and progeny in spikelet disarticulation. Each parental type produced a much larger percentage of progeny spikelets of its own kind of disarticulation than of the other type.

The 1922 data on the inheritance of spikelet disarticulation supported the conclusions drawn from the 1921 data on the inheritance of this character in Kherson. Eighteen progeny groups were grown in 1922 from parental florets disarticulating by semiabscission. Approximately 67 per cent of the progeny disarticulated in the same way. In only 4 of the 18 progeny groups was less than 50 per cent of the spikelet disarticulation by semiabscission, while in 5 strains more than 90 per cent, and in 1 group all progeny, were so classified.

Spikelets in which disarticulation was by fracture produced progenies in 1922 of which 81.4 per cent disarticulated by fracture and 18.6 per cent by semiabscission. The relation between parent and progeny for this character appears to be a definite one. In 8 of 21 groups sown from parental spikelets disarticulating by fracture spikelet disarticulation in all progeny was by fracture, while in only 3 of the other 13 groups did less than 50 per cent of the progeny separate by fracture. From these data it seems evident that two types of spikelet disarticulation exist in the Kherson variety. Both of these appear to be heritable to a large extent. It appears possible to isolate by selection pure-breeding lines of either type. Strains breeding true for spikelet disarticulation by fracture appear to be more numerous in Kherson than are those in which spikelet disarticulation is by semiabscission. It is impossible to determine the genetic constitution of Kherson on the basis of the data presented, but it appears evident that at least two factors are involved in the inheritance of spikelet disarticulation. The two methods of disarticulation in Kherson oat are shown in Plate 3.

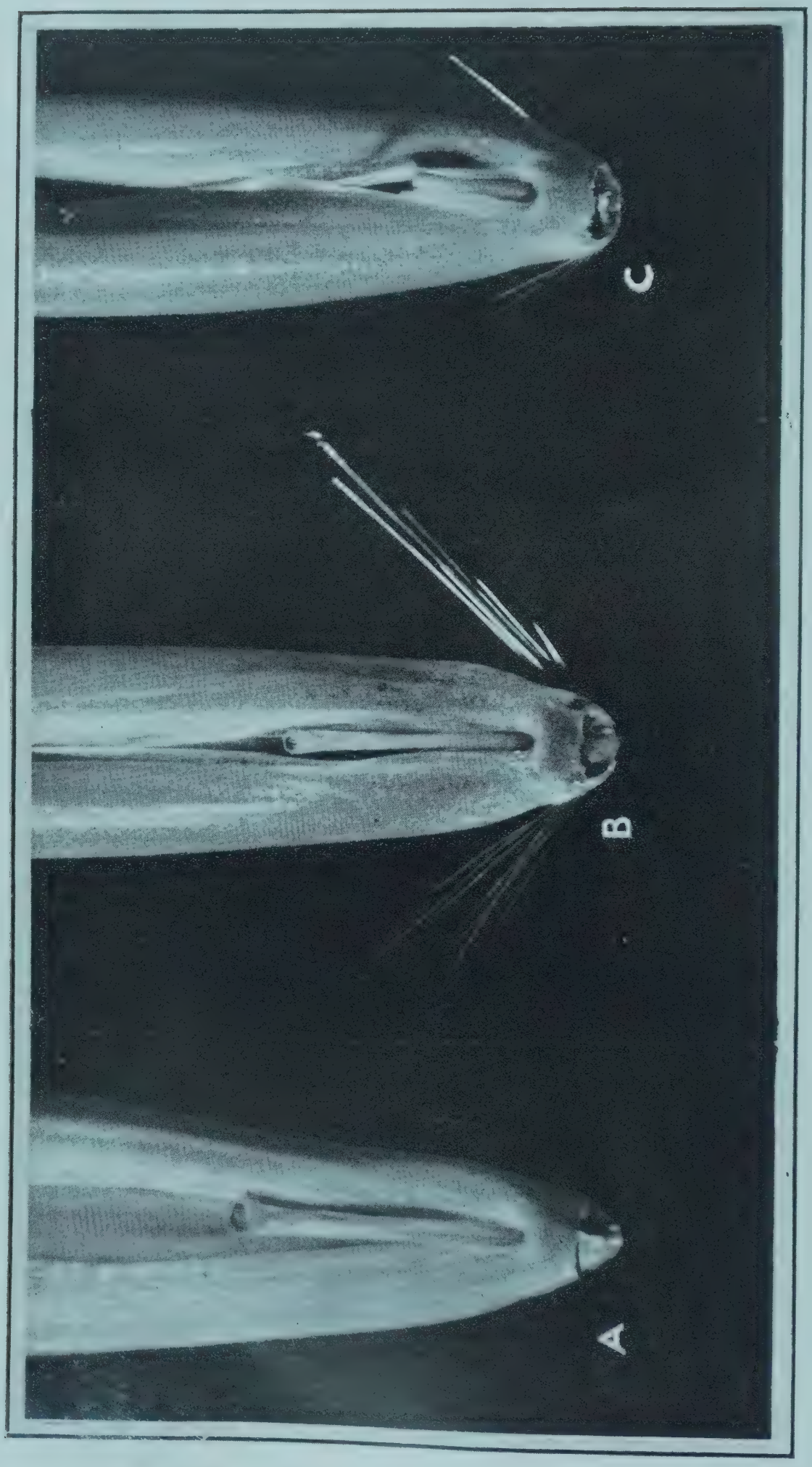
Of the 11 progeny groups grown in 1923 from parental kernels in which the spikelet disarticulated by fracture, 6 bred true and 2 groups produced progeny of which more than 95 per cent were like the parent. One group produced progeny of which nearly three-fourths disarticulated by semiabscission and one-fourth by fracture. Approximately 65 and 74 per cent, respectively, of the two remaining groups disarticulated the same in progeny as in parent.

Of the 9 groups of kernels in which spikelet disarticulation in the 1922 parental kernels was by semiabscission, 1 group produced progeny nearly all of which disarticulated by fracture, 2 produced progeny of which about 64 and 71 per cent, respectively, were of the parental description, 3 produced progeny of which approximately 95 per cent was like the parent, and in 3 groups all progeny were like the parent.

As a whole in 1923 spikelets in which disarticulation was by semiabscission produced progenies of which 77.8 per cent also disarticulated by semiabscission and 22.2 per cent by fracture. Of the parental kernels in which disarticulation was by fracture 85.9 per cent of the progeny was similar, only 14.1 per cent disarticulating by semiabscission. Compared with the data obtained the two previous seasons, progress toward homozygosity is clearly shown.

FLORET DISJUNCTION

Disjunction of the second florets from their supporting rachilla segments is one of the most important characters studied, as it is fundamental in the classification of Avena. The data on this character for 1921, 1922, and 1923 are shown in Table II. The floret disjunction in the 1921 crop was predominantly by disarticulation, although approximately 20 per cent of the progeny disjoined by heterofracture or by basifracture. Except for the 1922 data, it might have been believed that Kherson was heterozygous for floret disjunction. The 1922 and 1923 data are similar and show conclusively that the second florets of the Kherson variety separate by disarticulation, the common method in the species *Avena sativa*.



(For explanatory legend see p. 1073)

TABLE II.—*Inheritance of floret disjunction in strains of the Kherson oat grown at the Akron Field Station, Akron, Colo., in 1921, 1922, and 1923*

DATA FOR 1921

Floret disjunction of parent and group number	Floret disjunction in progeny					
	Number of kernels disjoining by—			Percentage of kernels disjoining by—		
	Basifracture	Heterofracture	Disarticulation	Basifracture	Heterofracture	Disarticulation
Disarticulation:						
1	1	10	75	1.2	11.6	87.2
2	2	34	42	2.6	43.6	53.8
3	1	1	11	7.7	7.7	84.6
4	----	3	16	-----	15.8	84.2
5	9	27	101	6.6	19.7	73.7
6	1	11	51	1.6	17.5	80.9
7	3	30	105	2.2	21.7	76.1
8	1	7	62	1.4	10.0	88.6
9	2	5	64	2.8	7.1	90.1
10	1	4	32	2.7	10.8	86.5
11	----	8	34	-----	19.0	81.0
12	2	27	81	1.8	24.6	73.6
13	1	7	54	1.6	11.3	87.1
14	1	29	160	.5	15.3	84.2
15	1	3	48	1.9	5.8	92.3
All groups	26	206	936	2.2	17.7	80.1

DATA FOR 1922

Heterofracture:						
33	----	6	69	-----	8.0	92.0
Disarticulation:						
1	1	18	204	0.4	8.1	91.5
2	----	3	102	-----	2.9	97.1
3	----	2	62	-----	3.1	96.9
4	----	2	82	-----	2.4	97.6
5	----	2	103	-----	1.9	98.1
6	----	19	55	-----	25.7	74.3
7	----	3	69	-----	4.2	95.8
8	----	2	155	-----	1.3	98.7
9	----	15	70	-----	17.6	82.4
10	----	----	69	-----	------	100.0
11	----	----	80	-----	------	100.0
12	----	5	95	-----	5.0	95.0
13	----	9	91	-----	9.0	91.0
14	----	----	30	-----	------	100.0
15	----	3	94	-----	3.1	96.9
16	----	----	26	-----	------	100.0
17	1	15	100	.9	12.9	86.2
18	----	10	103	-----	8.8	91.2
19	----	7	60	-----	10.4	89.6
20	----	13	72	-----	15.3	84.7
22	----	5	95	-----	5.0	95.0
23	----	6	58	-----	9.4	90.6
24	1	10	60	1.4	14.1	84.5
25	----	6	95	-----	5.9	94.1
26	----	8	70	-----	10.3	89.7

TABLE II.—*Inheritance of floret disjunction in strains of the Kherson oat grown at the Akron Field Station, Akron, Colo., in 1921, 1922, and 1923*—Continued

DATA FOR 1922—Continued

Floret disjunction of parent and group number	Floret disjunction in progeny					
	Number of kernels disjoining by—			Percentage of kernels disjoining by—		
	Basifracture	Heterofracture	Disarticulation	Basifracture	Heterofracture	Disarticulation
Disarticulation—Continued.						
27	----	----	56	-----	------	100.0
28	2	9	116	1.6	7.1	91.3
29	----	10	63	-----	13.7	86.3
30	1	7	67	1.4	9.3	89.3
31	----	7	109	-----	6.0	94.0
32	1	8	71	1.3	10.0	88.7
34	----	3	121	-----	2.4	97.6
35	----	4	54	-----	6.9	93.1
37	1	9	71	1.2	11.1	87.7
38	----	----	30	-----	------	100.0
39	----	----	17	-----	------	100.0
40	----	11	119	-----	8.5	91.5
41	----	1	74	-----	1.3	98.7
All groups	8	232	3,068	.3	7.0	92.7

DATA FOR 1923

Heterofracture:						
3	----	28	74	-----	27.5	72.5
15	----	2	50	-----	3.8	96.2
All groups	----	30	124	-----	19.5	80.5
Disarticulation:						
1	----	----	93	-----	------	100.0
2	----	9	116	-----	7.2	92.8
4	1	4	120	0.8	3.2	96.0
5	----	10	87	-----	10.3	89.7
6	----	8	61	-----	11.6	88.4
7	----	----	52	-----	------	100.0
8	----	4	132	-----	2.9	97.1
9	----	6	132	-----	4.3	95.7
10	1	9	115	.8	7.2	92.0
11	1	13	111	.8	10.4	88.8
12	----	12	97	-----	11.0	89.0
13	----	4	81	-----	4.7	95.3
14	----	1	42	-----	2.3	97.7
16	----	10	69	-----	12.7	87.3
17	----	5	120	-----	4.0	96.0
18	----	1	111	-----	.9	99.1
19	----	3	97	-----	3.0	97.0
20	----	----	78	-----	------	100.0
All groups	3	99	1,714	.2	5.5	94.3

EXPLANATORY LEGEND FOR PLATE 3

Spikelet disarticulation, floret disjunction, and basal hairs of Kherson oat

A. Spikelet disarticulation by fracture, floret disjunction by disarticulation, basal hairs absent
B. Spikelet disarticulation by semiabscission, floret disjunction by disarticulation, basal hairs abundant
C. Spikelet disarticulation by semiabscission, floret disjunction by heterofracture, basal hairs few

It is reasonable to assume that the comparatively small percentage of kernels in which floret disjunction was by basifracture and by heterofracture in all years can be accounted for by chance variations or as due to accidental causes in the breaking apart of the two kernels of the spikelet rather than to a hereditary cause. It is believed, therefore, that these data show conclusively that the Kherson oat used in these experiments belongs to the *Avena sativa* group in which floret disjunction usually is by disarticulation and that it contains few, if any, strains in which floret disjunction is by basifracture, as in the *A. sterilis* group. The different methods of floret disjunction are shown in Plates 3 and 4.

BASAL HAIRS

The results obtained on the inheritance of basal hairs are presented in Table III. These data show that parental florets classed as having few basal hairs produced progeny in 1921 of which about three-fourths had either few or abundant basal hairs and about one-fourth had the basal hairs absent. Parental florets with hairs absent produced progenies of which only about 40 per cent had hairs absent, while over 60 per cent had basal hairs present. These data indicate that a tendency exists for parental kernels having basal hairs to produce progeny having basal hairs. The fact that in 1921 parental kernels without hairs produced in many cases progeny having basal hairs either few or abundant may be accounted for by the fact that the original material was machine-threshed. As a result some parental kernels which originally bore basal hairs may have had them rubbed off in threshing (Table III).

The 1922 data show that 14 of the 25 groups of parental kernels described as having few basal hairs produced progeny largely or wholly of that description, one group having all progeny like the parents. In 9 of the remaining 11 groups more progeny kernels had basal hairs absent than bore few basal hairs, 3 of these groups showing no kernels with such hairs. In 2 groups about half of the progeny were of each class.

TABLE III.—*Inheritance of basal hairs in strains of the Kherson oat grown at the Akron Field Station, Akron, Colo., in 1921, 1922, and 1923*

DATA FOR 1921

Basal hairs of parent and group number	Basal hairs in progeny					
	Number of kernels having hairs			Percentage of kernels having hairs		
	Abundant	Few	Absent	Abundant	Few	Absent
Few:						
4	----	14	5	-----	73.7	26.3
6	3	44	16	4.8	69.8	25.4
9	----	40	31	-----	56.3	43.7
10	11	22	4	29.7	59.5	10.8
12	----	87	23	-----	79.1	20.9
15	----	52	-----	-----	100.0	-----
All groups	14	259	79	4.0	73.6	22.4
Absent:						
1	----	42	44	-----	48.8	51.2
2	2	48	28	2.6	61.5	35.9
3	----	3	10	-----	23.1	76.9
5	8	68	61	5.9	49.6	44.5
7	4	55	79	2.9	39.9	57.2
8	----	57	13	-----	81.4	18.6
11	----	11	31	-----	26.2	73.8
13	----	42	20	-----	67.7	32.3
14	----	155	35	-----	81.6	18.4
All groups	14	481	321	1.7	59.0	39.3

EXPLANATORY LEGEND FOR PLATE 4

Description of lemma color and other characters in Kherson florets

A. Yellowish white, basal hairs absent, awn twisted
B. Yellow, basal hairs absent, awn twisted
C. Reddish yellow, basal hairs absent, awn absent
D. White, basal hairs absent, awn nontwisted long
E. Yellowish white, spikelet disarticulation by fracture, floret disjunction by disarticulation
F. Yellow, spikelet disarticulation by fracture, floret disjunction by basifracture
G. Reddish yellow, spikelet disarticulation by fracture, floret disjunction by heterofracture, basal hairs absent, awn nontwisted long
H. White, spikelet disarticulation by fracture, floret disjunction by heterofracture, basal hairs absent, awn nontwisted short
I. Yellowish white, spikelet disarticulation by fracture, floret disjunction by disarticulation, basal hairs few, awn nontwisted long
J. Yellow, spikelet disarticulation by fracture, floret disjunction by disarticulation, basal hairs absent, awn absent
K. White, spikelet disarticulation by fracture, floret disjunction by disarticulation, basal hairs absent, awn absent
L. White, spikelet disarticulation by fracture, floret disjunction by disarticulation, basal hairs abundant, awn absent

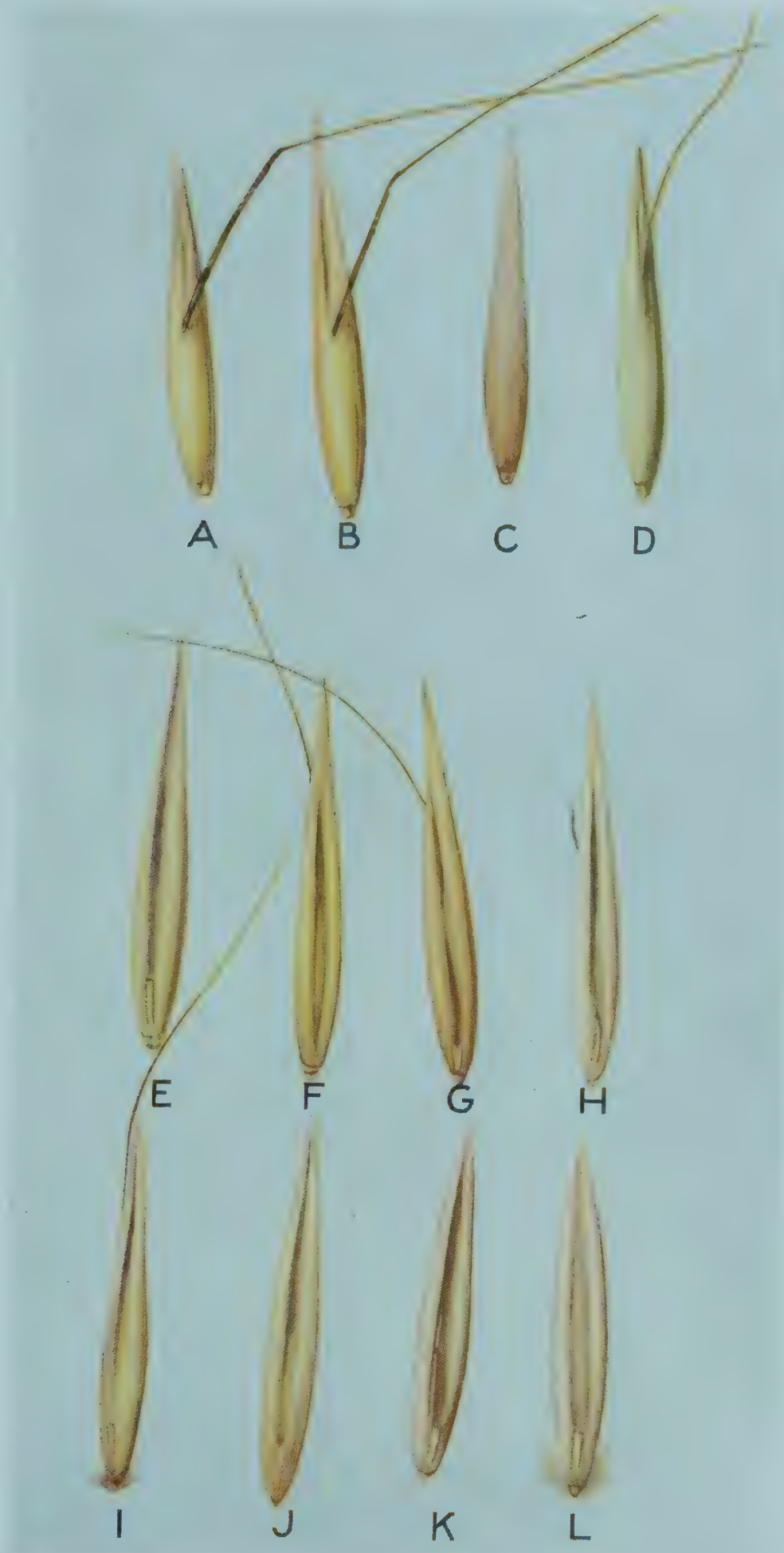

A
B
C
D
E
F
G
H
I
J
K
L

TABLE III.—*Inheritance of basal hairs in strains of the Kherson oat grown at the Akron Field Station, Akron, Colo., in 1921, 1922, and 1923*—Continued

DATA FOR 1922

Basal hairs of parent and group number	Basal hairs in progeny					
	Number of kernels having hairs			Percentage of kernels having hairs		
	Abundant	Few	Absent	Abundant	Few	Absent
Few:						
2		14	91		13.3	86.7
3		9	55		14.1	85.9
5		58	47		55.2	44.8
6		38	36		51.4	48.6
7			72			100.0
9		32	53		37.6	62.4
10			69			100.0
13		69	131		34.5	65.5
14		5	25		16.7	83.3
15			97			100.0
17	24	81	11	20.7	69.8	9.5
18		9	104		8.0	92.0
22		84	16		84.0	16.0
23		62	2		96.9	3.1
24		70	1		98.6	1.4
25		90	10		90.0	10.0
26		63	15		80.8	19.2
29	3	52	18	4.1	71.2	24.7
30		51	24		68.0	32.0
31		85	31		73.3	26.7
32		78	2		97.5	2.5
33		73	2		97.3	2.7
34		124			100.0	
35		55	3		94.8	5.2
37		76	5		93.8	6.2
All groups	27	1,278	920	1.2	57.4	41.4
Absent:						
1		5	218		2.2	97.8
4		22	62		26.2	73.8
8		3	154		1.9	98.1
11	4	2	74	5.0	2.5	92.5
12		35	65		35.0	65.0
16		2	24		7.7	92.3
19		7	60		10.4	89.6
20		30	56		34.9	65.1
27			56			100.0
28			127			100.0
38		2	28		6.7	93.3
39			17			100.0
40		1	129		.8	99.2
41			75			100.0
All groups	4	109	1,145	.3	8.7	91.0

DATA FOR 1923

Abundant:						
5		107	18		85.6	14.4
Few:						
11		116	9		92.8	7.2
14		25	18		58.1	41.9
15		44	8		84.6	15.4
16		78	1		98.7	1.3
18		112			100.0	
19		100			100.0	
20		53	25		67.9	32.1
All groups		528	61		89.6	10.4

TABLE III.—*Inheritance of basal hairs in strains of the Kherson oat grown at the Akron Field Station, Akron, Colo., in 1921, 1922, and 1923*—Continued

DATA FOR 1923—Continued

Basal hairs of parent and group number	Basal hairs in progeny					
	Number of kernels having hairs			Percentage of kernels having hairs		
	Abundant	Few	Absent	Abundant	Few	Absent
Absent:						
1		12	81		12.9	87.1
2		86	39		68.8	31.2
3		39	63		38.2	61.8
4		8	117		6.4	93.6
5		47	50		48.5	51.5
6		26	43		37.7	62.3
7		11	41		21.2	78.8
8		10	126		7.4	92.6
9		9	129		6.5	93.5
12		5	104		4.6	95.4
13		39	46		45.9	54.1
17			125			100.0
All groups		292	964		23.2	76.8

Of the 14 groups of the 1922 crop having parental kernels with basal hairs absent all progenies in four groups had no hairs. In only four groups were found florets having basal hairs to any considerable degree. This shows that a strong tendency exists for parental kernels without hairs to produce progeny of that description, while parental kernels with hairs show a marked tendency to produce progeny which segregate into the few and absent classes for basal hairs.

The comparatively few kernels which were described as having abundant basal hairs in this study may easily be accounted for as chance variations in all cases. There seemed to be one exception to this tendency, group 17 of the 1922 crop, but the results obtained in 1923 indicated that these few kernels also were due to chance variation and not to any heritable difference.

The data obtained in 1923 in the study of basal hairs in progeny of kernels bearing few hairs indicate that this character is more definitely inherited than was shown by similar studies in previous years. Between 85 and 90 per cent of the progeny from parental kernels bearing hairs was described as bearing hairs. However, in 11 of the 12 groups in which the parental kernels were described as having no hairs some progeny kernels were pro-

duced which bore a few hairs. Although, in 1922, 91 per cent of the progeny of kernels with hairs absent was described as without hairs, in 1923 only 76.8 per cent of the progeny of parental kernels without hairs was so described. The fluctuations in the percentages of true breeding progeny from parental kernels described as having basal hairs absent probably indicates that this character is influenced by environmental or physiological factors.

The results, however, apparently indicate the possibility of isolating strains of Kherson oats which will breed comparatively pure for few as well as for no basal hairs. Presumably pure-breeding strains having few basal hairs are as numerous in the Kherson variety as grown at Akron as those having no basal hairs. The variation in length and abundance of basal hairs found in Kherson oats is shown in Plates 2 and 4.

AWNS

The present study was conducted chiefly to obtain data on the inheritance of awning in Kherson oats. Nilsson-Ehle (*16*) apparently first stated the belief that yellow color had an inhibitory effect on awn development. The writers may be misled, but it appears that this theory possibly was advanced on the results observed by Nilsson-Ehle in a cross in which less than 25 plants with yellow kernels were produced in the F_2 generation, and of these few progeny plants some were awned. The results obtained by Surface (*20*) in his cross of *Avena fatua* with the cultivated variety Kherson did not support this theory of Nilsson-Ehle.

The results of Love and Fraser (*13*), Love and Craig (*14*), and Fraser (*6*) all fail to show conclusively that the Kherson or Sixty-Day oat contains an inhibitor for awns linked with yellow color. The failure to observe the expected inhibitory effect is explained by these writers largely on the basis that the Burt oats used in their crosses carried no such inhibitor, and thus obscured the effect of the inhibitor carried by the Kherson or Sixty-Day variety. They further explain their failure to obtain an inhibitory effect from Sixty-Day in their crosses of Sixty-Day × Burt as being partially due to climatic conditions, which may favor the production of awns in one season or cause kernels which carry the gene for awns to fail to produce awns in other seasons.

The results obtained in the present experiment do not indicate either the linkage of awnlessness and yellow color or the inhibitory effect of yellow color for the production of awns in the Kherson variety.

The data obtained in the study of awns in the Kherson oat in 1921, 1922, and 1923 are presented in Table IV.

Parental florets having twisted awns produced progenies in 1921, of which about 48 per cent had twisted, 20 per cent had nontwisted long, 2 per cent nontwisted short awns, and 32 per cent was awnless. Parental florets having nontwisted long awns produced progenies of which 7.5 per cent had twisted awns, 45.2 per cent nontwisted long, 1.4 per cent nontwisted short, and 45.9 per cent had absent awns. Awnless parental florets produced progeny of which about 90 per cent were awnless, 7 per cent had nontwisted long awns, 1.6 per cent had nontwisted short awns, and 1.8 per cent had twisted awns. The data obtained in 1921 indicated that the twisted, nontwisted long, and absent awns differed in their hereditary behavior. It appeared that the absence of awns was more constant in breeding behavior than the others and that the nontwisted long awn differs genetically from the twisted awn. Both types appeared similar in the production of florets with nontwisted short or absent awns.

Much more definite data were obtained on the inheritance of awns in Kherson oats in 1922. These data indicate that the twisted awn possibly is recessive to the nontwisted long awns and to awnlessness. Nearly 95 per cent of the progeny of parental florets having twisted awns had twisted awns, while a very few had nontwisted long or short awns, and only about 5 per cent was awnless. The studies in Burt oats by Coffman, Parker, and Quisenberry (*4*) have shown the tendency of kernels in that variety having the twisted awn to produce progeny with awns of that character or no awns at all. Parental kernels of Kherson oats having nontwisted long awns produced progeny in 1922 of which 64.9 per cent had nontwisted long awns, 12 per cent had twisted awns, 10.1 per cent had nontwisted short awns, and 13 per cent was awnless.

The failure of awnless parental kernels to produce progeny all of which were awnless has been noted by previous investigators. In the 1922 crop of Kherson oats grown at Akron 74.3 per cent of the progeny of awnless parental kernels was awnless, 5.8 per cent had nontwisted short awns, 18.7 per cent had nontwisted long awns, and 1.2 per cent had twisted awns.

TABLE IV.—*Inheritance of awns in strains of the Kherson oat grown at the Akron Field Station, Akron, Colo., in 1921, 1922, and 1923*

DATA FOR 1921

Awn of parent and group number	Awns in progeny							
	Number of kernels having awns				Percentage of kernels having awns			
	Twisted	Nontwisted		Absent	Twisted	Nontwisted		Absent
		Long	Short			Long	Short	
Twisted:								
4		8		11		42.1		57.9
5	96			41	70.1			29.9
8	61	4	3	2	87.1	5.7	4.3	2.9
12	38	35	2	35	34.6	31.8	1.8	31.8
14	31	62	6	91	16.3	32.6	3.2	47.9
15	48	2		2	92.3	3.9		3.8
All groups	274	111	11	182	47.4	19.2	1.9	31.5
Nontwisted:								
3	6	1		6	46.2	7.7		46.1
9		40	1	30		56.3	1.4	42.3
13	5	25	1	31	8.1	40.3	1.6	50.0
All groups	11	66	2	67	7.5	45.2	1.4	45.9
Absent:								
1		9	2	75		10.5	2.3	87.2
2				78				100.0
6	5	15	5	38	7.9	23.8	7.9	60.4
7		6		132		4.3		95.7
10				37				100.0
11	3	1		38	7.1	2.4		90.5
All groups	8	31	7	398	1.8	7.0	1.6	89.6

DATA FOR 1922

Twisted:								
7	65			7	90.3			9.7
8	155			2	98.7			1.3
10	69				100.0			
11	78			2	97.5			2.5
14	29			1	96.7			3.3
15	86			11	88.7			11.3
22	90	1	1	8	90.0	1.0	1.0	8.0
23	64				100.0			
34	120	1	1	2	96.8	.8	.8	1.6
37	67	2	3	9	82.7	2.5	3.7	11.1
All groups	823	4	5	42	94.1	.5	.6	4.8
Nontwisted:								
16		22	2	2		84.6	7.7	7.7
24	3	64	1	3	4.2	90.2	1.4	4.2
25	21	63	9	7	21.0	63.0	9.0	7.0
26	29	38	6	5	37.2	48.7	7.7	6.4
29		42	17	14		57.5	23.3	19.2
31	21	60	9	26	18.1	51.7	7.8	22.4
32	12	63	5		15.0	78.8	6.2	
33		74		1		98.7		1.3
38		10	20			33.3	66.7	
41	1	34	4	36	1.4	45.3	5.3	48.0
All groups	87	470	73	94	12.0	64.9	10.1	13.0

TABLE IV.—*Inheritance of awns in strains of the Kherson oat grown at the Akron Field Station, Akron, Colo., 1921, 1922, and 1923*—Continued

DATA FOR 1922—Continued

Awn of parent and group number	Awns in progeny							
	Number of kernels having awns				Percentage of kernels having awns			
	Twisted	Nontwisted		Absent	Twisted	Nontwisted		Absent
		Long	Short			Long	Short	
Absent:								
1		66	25	132		29.6	11.2	59.2
2	3	47	15	40	2.9	44.7	14.3	38.1
3				64				100.0
4				84				100.0
5				105				100.0
6	4	1		69	5.4	1.4		93.0
9	3	1		81	3.5	1.2		95.2
12			1	99			1.0	99.3
13				200				100.0
17				116				100.0
18		30	10	73		26.5	8.9	64.0
19			7	60			10.4	89.6
20	3	60	8	15	3.5	69.8	9.3	17.6
27		22	11	23		39.3	19.6	41.4
28	5	26	5	91	3.9	20.5	3.9	71.1
30		21	12	42		28.0	16.0	56.7
35	3	8	2	45	5.2	13.8	3.4	77.6
39	1	15		1	5.9	88.2		5.9
40		55	14	61		42.3	10.8	46.9
All groups	22	352	110	1,401	1.2	18.7	5.8	74.3

DATA FOR 1923

Twisted:								
4	124			1	99.2			0.8
9	137			1	99.3			.7
15	34	14	2	2	65.4	26.9	3.9	3.8
18	59	17	15	21	52.7	15.2	13.4	18.7
19	72	11	2	15	72.0	11.0	2.0	15.0
20	18	11	3	46	23.1	14.1	3.8	59.0
All groups	444	53	22	86	73.4	8.8	3.6	14.2
Nontwisted:								
1	2	29	9	53	2.2	31.2	9.7	56.9
14	8	21	11	3	18.6	48.8	25.6	7.0
16	10	66	1	2	12.7	83.5	1.3	2.5
17		18	3	104		14.4	2.4	83.2
All groups	20	134	24	162	5.9	39.4	7.1	47.6
Absent:								
2			1	124			.8	99.2
3	3		1	98	2.9		1.0	96.1
5	7	5	3	82	7.2	5.2	3.1	84.5
6				69				100.0
7			1	51			1.9	98.1
8				136				100.0
10				125				100.0
11				125				100.0
12		1	1	107		.9	.9	98.2
13		2	1	82		2.3	1.2	96.5
All groups	10	8	8	999	1.0	.8	.8	97.4

The data obtained on the inheritance of awns in the Kherson oat in 1923 were similar to those of previous seasons in many respects. Although the percentage of progeny of kernels described as bearing twisted awns was smaller in 1923 than in 1922, it was much larger than that of 1921. The breeding behavior of the kernels having nontwisted long awns was very similar in 1923 to that of 1921, and awnless parental kernels indicated a very strong tendency to produce only awnless progeny.

The data on inheritance of awns clearly indicate that genetically three types of awns exist in the Kherson oat. All three types tend to breed true. The twisted awn appears to breed as a recessive. Possibly because of physiological influences, some kernels which carry the factor for producing this type of awn fail to produce awns at all or produce the other types. The nontwisted long awn in Kherson is much less definite in breeding behavior than is the twisted awn. The nontwisted short awn in Kherson probably results from chance variation. Its genetic constitution probably is similar to the nontwisted long awn. It appears probable that pure-breeding strains which bear long awns can be isolated from the variety. The data for each group show more clearly than do the summaries for all groups that many pure-breeding awnless strains exist in Kherson oat, although not all awnless strains breed true for that condition.

Nilsson-Ehle (*17*) has stated that external conditions may greatly influence the production of awns in cultivated oat varieties. The junior author in classification studies of oats conducted at widely separated points in the United States has observed that the number of awns in some varieties and strains varies apparently with environmental conditions. For this reason it can not be assumed that these Kherson strains would show a similar behavior for awns under a decidedly different set of conditions.

The awn types found in the present study of Kherson oats are shown in Plates 2 and 4.

LEMMA COLOR

Only three lemma colors have been recognized in this study of the Kherson variety. These colors, reddish yellow, yellow, and white, have been described previously (pl. 4). Table V presents the data on color in 1921, 1922, and 1923, and shows conclusively that the yellow color is the most stable in breeding behavior. Both of the other kernel colors tend to break up and produce more progeny described as yellow than of the other types. The distinctions between the different color classes in oats are often difficult to make, as the colors grade into one another. This is due partly to the effects of physiological influences, which may cause genetically white kernels to be called yellow or yellow kernels to appear either reddish yellow or white.

TABLE V.—*Inheritance of lemma color in strains of the Kherson oat grown at the Akron Field Station, Akron, Colo., in 1921, 1922, and 1923*

DATA FOR 1921

Color of parental lemmas and group number	Lemma color in progeny					
	Number of lemmas			Percentage of lemmas		
	Reddish yellow	Yellow	White	Reddish yellow	Yellow	White
Reddish yellow:						
8	3	45	12	5.0	75.0	20.0
Yellow:						
1	11	69	6	12.8	80.2	7.0
9	5	66	----	7.0	93.0	-----
10	25	12	----	67.6	32.4	-----
11	9	30	3	21.4	71.4	7.2
12	34	76	----	30.9	69.1	-----
13	1	54	7	1.6	87.1	11.3
14	78	112	----	41.1	58.9	-----
15	45	7	----	86.5	13.5	-----
All groups	208	426	16	32.0	65.5	2.5
White:						
2	----	44	34	------	56.4	43.6
3	6	7	----	46.2	53.8	-----
4	1	10	----	9.1	90.9	-----
5	4	19	97	3.4	15.8	80.8
6	18	27	4	36.7	55.1	8.2
7	2	136	----	1.4	98.6	-----
All groups	31	243	135	7.6	59.4	33.0

DATA FOR 1922

Reddish yellow:						
2	6	99	----	5.7	94.3	-----
17	----	116	----	------	100.0	-----
22	3	97	----	3.0	97.0	-----
24	----	71	----	------	100.0	-----
27	----	56	----	------	100.0	-----
31	8	105	3	6.9	90.5	2.6
32	2	78	----	2.5	97.5	-----
33	----	75	----	------	100.0	-----
37	9	72	----	11.1	88.9	-----
All groups	28	769	3	3.5	96.1	.4
Yellow:						
1	20	203	----	9.0	91.0	-----
3	----	64	----	------	100.0	-----
4	44	40	----	52.4	47.6	-----
12	----	43	57	------	43.0	57.0
13	----	155	45	------	77.5	22.5
15	17	64	16	17.5	66.0	16.5

TABLE V.—*Inheritance of lemma color in strains of the Kherson oat grown at the Akron Field Station, Akron, Colo., in 1921, 1922, and 1923*—Con.

DATA FOR 1922—Continued

Color of parental lemmas and group number	Lemma color in progeny					
	Number of lemmas			Percentage of lemmas		
	Reddish yellow	Yellow	White	Reddish yellow	Yellow	White
Yellow—Contd.						
16	1	25	----	3.8	96.2	-----
18	11	102	----	9.7	90.3	-----
19	41	26	----	61.2	38.8	-----
20	2	84	----	2.3	97.7	-----
23	----	64	----	------	100.0	-----
25	----	100	----	------	100.0	-----
26	----	78	----	------	100.0	-----
28	----	127	----	------	100.0	-----
29	----	73	----	------	100.0	-----
30	4	71	----	5.3	94.7	-----
34	31	93	----	25.0	75.0	-----
35	----	58	----	------	100.0	-----
38	2	28	----	6.7	93.3	-----
39	----	17	----	------	100.0	-----
40	----	130	----	------	100.0	-----
41	----	75	----	------	100.0	-----
All groups	173	1,720	118	8.6	85.5	5.9
White:						
5	----	105	----	------	100.0	-----
6	42	32	----	56.8	43.2	-----
7	20	37	15	27.8	51.4	20.8
8	6	81	70	3.8	51.6	44.6
9	4	55	26	4.7	64.7	30.6
10	2	54	13	2.9	78.3	18.8
11	----	34	46	------	42.5	57.5
14	12	18	----	40.0	60.0	-----
All groups	86	416	170	12.8	61.9	25.3

DATA FOR 1923

Reddish yellow:						
3	----	------	102	------	------	100.0
13	----	85	----	------	100.0	-----
All groups	----	85	102	------	45.5	54.5
Yellow:						
1	----	93	----	------	100.0	-----
2	----	44	81	------	35.2	64.8
4	----	------	125	------	------	100.0
5	----	------	97	------	------	100.0
6	----	19	50	------	27.5	72.5
7	----	------	52	------	------	100.0
8	----	118	18	------	86.8	13.2
10	----	125	----	------	100.0	-----
11	----	125	----	------	100.0	-----
12	----	109	----	------	100.0	-----
14	----	43	----	------	100.0	-----
15	----	52	----	------	100.0	-----
16	----	79	----	------	100.0	-----
17	----	125	----	------	100.0	-----
18	----	112	----	------	100.0	-----
19	----	100	----	------	100.0	-----
20	----	78	----	------	100.0	-----
All groups	----	1,222	423	------	74.3	25.7
White:						
9	----	------	138	------	------	100.0

The parental groups in which the lemma color was described as reddish yellow in 1922 produced progenies of which over 96 per cent of the lemmas was yellow. White parental kernels produced progenies in which about 62 per cent of the lemmas was yellow and 13 per cent reddish yellow. Parental kernels classed as yellow produced yellow progeny very largely.

It would be difficult to explain on a factorial basis the data on lemma color shown in Table V. All classes tended to produce a high percentage of yellow kernels. Most of the reddish-yellow lemmas in Kherson apparently are due to physiological causes. The white kernels appeared unstable in breeding behavior in 1921 and 1922, as they produced many of the yellow and reddish-yellow types. In 1923, however, parental white kernels produced only white progeny. Probably many genetic yellows were incorrectly described as white in the 1921 crop, due to physiological influences. This condition is very evident in the progeny of groups 5, 6, and 14, from which no white kernels whatever were produced in 1922. The parent kernels, apparently, were simply bleached yellow.

These data indicate that at least two genetic color types exist in the Kherson oat—yellow and white. The yellow type apparently is decidedly more numerous and also less influenced by physiological factors than the white. Possibly some of the yellows are heterozygous, which would account for the occurrence of white kernels in the progenies of parental kernels described as yellow. Physiological factors may account for some of the progeny of white kernels being described as yellow and reddish yellow in 1921 and 1922.

SUMMARY

The Kherson and Sixty-Day oats are identical for all characters. Together with the pure-line selections developed from them, they constitute a very important agronomic group, occupying about 14 per cent of the oat acreage in the United States in 1919.

Several valuable selections have been made from the variety, among which Albion (Iowa No. 103), Richland (Iowa No. 105), Iowar, Nebraska No. 21, and States Pride (Wisconsin No. 7), are the most important.

The variability of the variety and the possibility of making selections from it have long been recognized.

Numerous crosses using strains of the variety have been made, and the genetic constituton of some of the result-

ant strains has been rather definitely stated. Only a few of these crosses have produced strains of any economic promise.

Spikelet disarticulation by abscission, so characteristic of Red Rustproof oats, does not occur in Kherson. Disarticulation by semiabscission breeds true in some strains, but shows apparent segregation in others. Most strains show the more or less rough and pointed base resulting from fracture, which is characteristic of *Avena sativa* varieties.

Floret disjunction in strains of Kherson oat is predominantly by disarticulation, as in *Avena sativa*, and probably is homozygous. The very small percentage of kernels showing floret disjunction either by basifracture, as in *A. sterilis*, or by heterofracture, an intermediate type, can be explained on the principle of chance variation.

Basal hairs appear exceedingly complex in their mode of inheritance. With few exceptions all basal hairs in the Kherson oat are midlength to short. Several factors apparently are involved and further study is necessary to determine their true genetic behavior.

The inheritance of awn type apparently shows no definite relation between the presence of awns and kernel color. This is contrary to the results obtained by certain other investigators. The data obtained in the present investigations indicate that the yellow color of the Kherson strain used does not carry an inhibitor for awns, as all types of awns have been found on yellow kernels. It has been shown further that it is possible to isolate yellow-kerneled strains which apparently are homozygous for certain awn types as well as for practically complete awnlessness. Several factors apparently are concerned in the production of awns in Kherson oats. Further investigations are necessary to determine the genotypic constitution of strains having different awn types and the influence of physiological factors on awn production.

Apparently only two factors for color exist in Kherson oat lemmas—viz., one for yellow and one for white. Some strains evidently are heterozygous for lemma color, but yellow is predominant, and many yellow strains are homozygous, as shown by their breeding behavior. Some white strains exist in Kherson, but they are much less numerous. Apparently physiological influences often cause genetically yellow kernels to be classed as phenotypically white kernels and vice versa.

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RELATION BETWEEN WEATHER CONDITIONS AND YIELD OF COTTON IN LOUISIANA [1]

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INTRODUCTION

This study was undertaken to discover to what degree the variations in yield of cotton may be explained on the basis of the available weather data. Obviously the yield of cotton must be greatly influenced by factors which operate prior to our being aware of their effect as measured by the resulting yield. If we may measure these factors as they occur, and then discover a statistical relation between the antecedent conditions and the subsequent yields, we are then in position to make forecasts of yield at the time the weather conditions occur. In the following discussion it will be shown that a fairly close relationship was established between the weather conditions prior to September and the subsequent final yield of cotton. The nature of these functional relations is shown in Figure 1.

Analyses of the quantitative relationship between weather factors and yields of crops have been made by several investigators. For example, H. L. Moore in 1917,[2] and more recently J. Warren Smith, of the United States Weather Bureau,[3] have both published results of such investigations. The present contribution is an extension of the methods suggested in Moore's book. A statement follows of the theoretical considerations upon which the subsequent statistical analysis was made.

FACTORS INFLUENCING YIELD

There are four major types of factors influencing yield: (1) Inherent capacity of the plant itself to bear; (2) fertility of the soil; (3) weather and length of the growing season; and (4) cultural methods applied to the crop during its growth; that is, the influence which the grower can exert to accentuate favorable, and minimize unfavorable, natural conditions.

If a State is considered as a whole, the influence of some of these factors, while continuing to be of significance in determining the long-time or trend movement in yield, is of little significance in year-to-year variations. There is great inertia in the cultural habits of the producer, for example. A new method when introduced is adopted and tried by a few, and if successful it is taken up by others the following season. Such a slow change is witnessed in the gradual introduction of the practice of spacing plants closely in the row—from 4 to 8 inches—a practice that is recommended by the United States Department of Agriculture as a means of reducing losses from weevil. The effects of such changes, even if marked in given localities, are but slight when the State is considered as a whole and the total effect on yield is so spread over a number of years that it may be considered as a long-time or "trend" change and conversely of little importance in year-to-year variations.

A similar proposition holds true for seed introduction. The shifting from one staple length to another in accordance with the premiums offered for long staple cotton will, however, probably find some reflection in yield, since a normal yield in pounds per acre for short staple is considerably greater than for long staple. Consequently, variations in yield not accounted for by weather may be considered in some degree attributable to this factor.

Soil fertility of the areas in cotton may be changed by the shifting of acreages, the opening up of new lands, and gradual exhaustion of the soil. The producer may also apply various kinds and amounts of fertilizer according to his cultural practice. A glance at Figure 1 will show that there is a distinct downward trend in yield. The bringing in of new lands less suitable for cotton growing may in part explain this trend, but it is more probable that the Mexican boll weevil has more to do with it. As in

[1] Received for publication Sept. 15, 1924; issued August, 1925.

[2] MOORE, H. L. FORECASTING THE YIELD AND THE PRICE OF COTTON. 173 p., illus. New York. 1917.

[3] SMITH, J. W. INFLUENCE OF THE WEATHER ON THE YIELD OF CROPS. Mo. Weather Rev. 50: 567–572, illus. 1922.

the case of the year-to-year changes in staple length, so the changes in the amounts of fertilizer used are of some, but probably small, importance in accounting for the variations in yield. If the bringing in of new lands has a significant effect on fertility, this too may be considered as a long-time or trend influence. Weather as a factor in yield may now be considered.

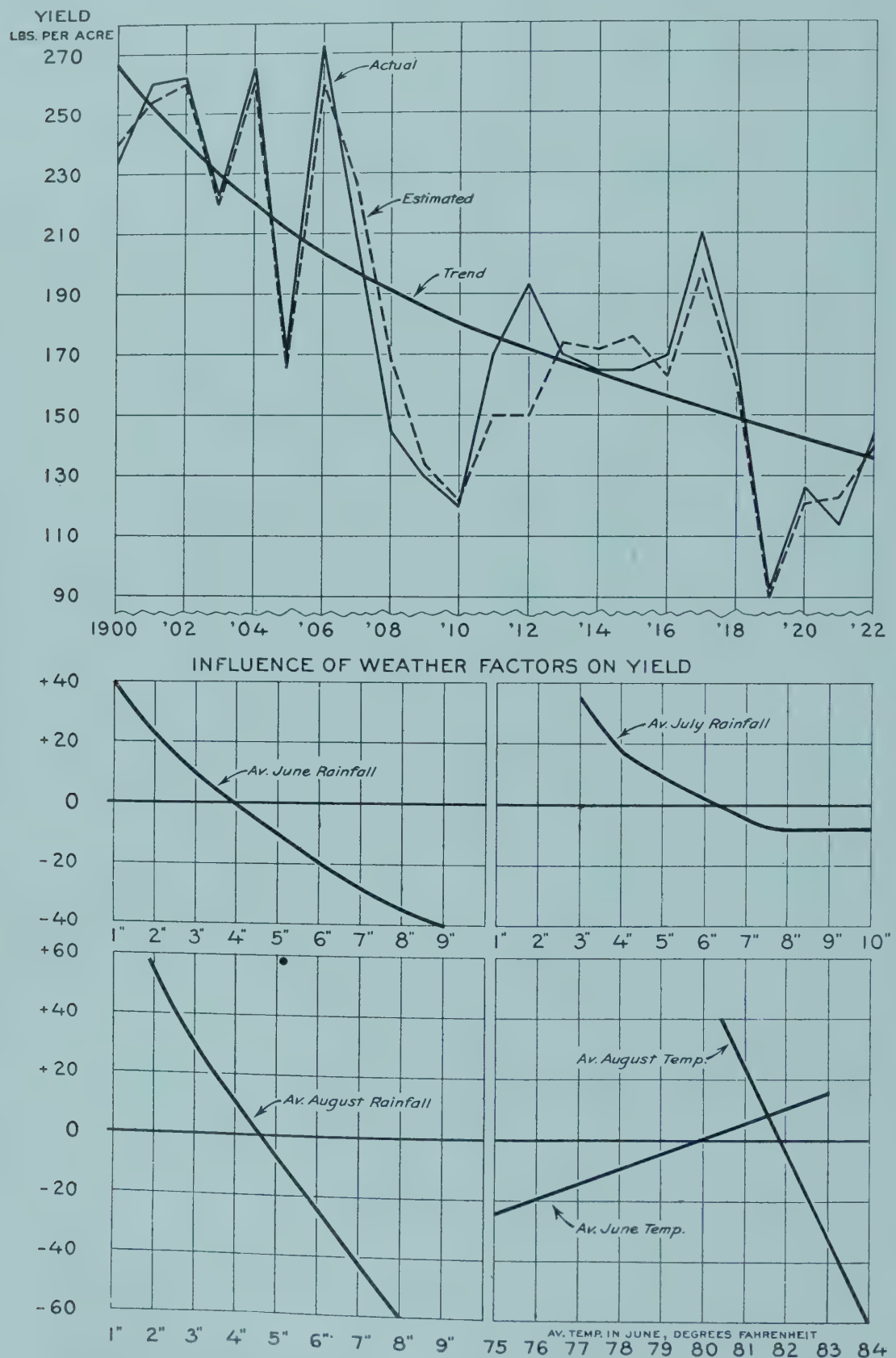

FIG. 1.—Yield of cotton in Louisiana and yield estimated from weather factors, 1900–1922

The cotton plant can not survive in a freezing temperature, and it is conceivable that a temperature could be too high. Somewhere between the extremes there must be an optimum point. There is probably also an optimum condition of rainfall. Since there is considerable variation in rainfall and temperature from one season to another, there must necessarily be considerable deviation from this optimum. As weather shows the greatest year-to-year variation of the four factors mentioned and is of influence throughout the entire State, there is presumably a relation between changes in weather conditions and changes in average yield. The attempt was accordingly made to discover this relation between weather conditions and yield, and to ascertain to what degree the latter may be explained by the former. Residual variations in yield (changes left over after the effects of weather have been eliminated) were then considered attributable to one or more of three things: (1) Errors in the original estimates of yield and weather data used; (2) an insufficient statistical expression of weather conditions; and (3) influence of the factors mentioned other than weather. As a matter of fact, these residuals were very slight, thus furnishing statistical support for the hypothesis advanced.

TABULATION OF DATA

For use in the quantitative analysis, data on yield per acre and rainfall and temperature were selected. Official figures of yield per acre of the United States Department of Agriculture were selected after comparison with the sources from which the final figures are taken. This is an expression of yield in pounds of lint cotton produced per acre.

Selection of weather data to be used was governed to a considerable extent by what is available without reworking the original Weather Bureau records. Undoubtedly, percentage of sunshine, humidity, winds, rainfall, and temperature taken for short periods of time, particularly in critical growing periods, are of importance. As only monthly data, however, are readily available over the period of time selected, 1900 to 1922, inclusive, the shortest weather period that could be used was automatically defined. Furthermore, temperature and rainfall are the only satisfactory State average weather figures covering the period. Accordingly the following series (Table I) for the State selected (Louisiana) were chosen for comparison and correlation with yield.

A number of other rainfall and temperature series were experimented with, but they showed slight relation to yield and are not included here. Table I records the original data described in the preceding.

TABLE I.—*Average rainfall and temperature for certain months and yield of lint cotton per acre, Louisiana, for the years 1900 to 1922 inclusive.*

Year	June	July	August	June	August	Yield
	Inches	*Inches*	*Inches*	*°F.*	*°F.*	*Pounds*
1900	8.40	7.11	4.59	79.3	81.4	234
1901	2.99	7.08	5.46	81.2	81.9	260
1902	1.84	4.56	3.47	81.6	84.1	262
1903	4.18	6.12	4.71	75.5	81.7	223
1904	3.89	7.17	5.19	80.0	80.2	265
1905	7.22	8.52	4.92	81.1	82.6	170
1906	3.40	7.97	2.92	81.8	82.1	272
1907	1.77	4.07	4.66	79.2	82.8	210
1908	4.10	10.61	6.61	80.6	81.3	145
1909	6.51	3.90	6.12	80.2	82.8	130
1910	7.26	7.85	4.42	78.1	82.8	120
1911	4.63	9.37	8.01	82.7	81.5	170
1912	5.67	6.63	5.89	77.3	81.3	193
1913	3.72	7.19	4.47	78.4	81.7	170
1914	2.51	7.07	7.48	83.4	80.8	165
1915	3.11	5.75	6.78	81.9	80.8	165
1916	3.89	8.00	4.49	80.5	82.0	170
1917	1.29	5.58	5.71	79.5	81.1	210
1918	3.70	3.16	6.68	82.7	81.9	167
1919	6.63	6.10	5.11	78.3	82.8	93
1920	5.21	8.18	6.61	78.7	80.5	126
1921	5.42	6.60	3.31	80.3	83.4	114
1922	5.61	5.85	5.26	80.4	81.3	144

The method of handling the described data is an extension of multiple correlation methods [4] designed by M. Ezekiel, of the Bureau of Agricultural Economics, and is briefly as follows:

By the usual methods of multiple correlation,[5] the net regression lines showing net effect of each weather factor upon the yield are plotted. The values of the dependent as obtained from the regression equation are determined, as are the residuals—plus or minus differences between the actual yield and yield estimated from regression equation. These residuals are in turn plotted against each weather factor as deviations from each of the regression lines, the lines then being curved to pass through the plotted points in so far as consistent with the hypothesis of a "smooth curve" func-

[4] EZEKIEL, M. J. B. A METHOD OF HANDLING CURVILINEAR CORRELATION FOR ANY NUMBER OF VARIABLES. Jour. Amer. Statis. Assoc. 19: 431-453, illus., 1924.

[5] TOLLEY, H. R., and EZEKIEL, M. J. B. A METHOD OF HANDLING MULTIPLE CORRELATION PROBLEMS. Jour. Amer. Statis. Assoc. 18: 993-1003. 1923.

tion. From these curves the dependents are again estimated by reading from the curve the yield value associated with each given weather value. New residuals are obtained and plotted as deviations from the regression curves, and the process is continued until the residuals can not be reduced further. It is seen that this method is one of approximation and as such is not susceptible to the mathematical demonstration of validity and probability as are many other statistical methods. On the other hand, as measured by the closeness with which the actual yield may be estimated from the independent factors and by empirical tests, the method is considerably superior. Linear correlation is not a satisfactory method of analysis when the theory predicates nonlinear relationships, that is, optimum conditions. For the purpose of obtaining the best trend line, time was treated as an independent variable in this analysis.

When the curvilinear analysis just described is completed, the net functional relationship between the weather factors and yield is described by the resulting curves. These curves are charted in Figure 1. By reading from them the yield deviations from trend (ordinates) associated with the given weather factors measured in any given season (abscissae), and by summing these readings and subtracting the constant, 2.0, an estimate of the yield may be obtained, expressed as deviation from normal or trend value. This can be done early in September, thus giving an unusually close estimate of the final yield at a comparatively early date. Such estimates were worked out for the period studied, and the curve so obtained is shown on the chart. With the omission of two years, 1911 and 1912, where there is a most pronounced recovery in the yield figure, the correlation index [6] between the actual and the estimated is unusual (0.97), the standard error of estimate being but a fourth of the standard deviation of yield from trend.

Two considerations tend to diminish the reliability of forecasts made from these curves. In the first place there are very few observations upon which to base the curves. There are about 20 years which have been used to establish five separate functional relationships. The correlation is probably in some degree due to the mere numerical probabilities of adjusting these functions to observations. This influence, however, is probably slight, since the correlation is so unusually high and since the curves represent relationships which are in accordance with the conception of what the effect of weather factors should be. For example, the curves indicate that there is generally too much rain during the June-to-August growing period, since an increase in rainfall is accompanied by a decrease in yield. This furnishes support for the proposition that an increase in rainfall increases the activity of the weevil and hence increases crop damage.

In the second place, during the first few years of the period covered there was but slight boll-weevil infestation. In 1909, however, practically all of Louisiana was infested. The weather is considered an important factor in the life of the weevil. Thus what would normally be good weather for cotton growing might be the reverse under infestation, because such weather might favor weevil propagation; that is, the influence of weather factors when operating on the cotton plant through the weevil would be different from the influence when these factors were operating directly upon the plant. The curves, then, contain the results of two mixed influences, one of which is now predominant. To avoid this mixture through shortening the period by limiting the analysis to those years subsequent to 1908 is to so greatly increase the first possibility of error mentioned as to render any curves practically invalid. But as an experiment, a linear multiple correlation coefficient was obtained for the years 1908 to 1922, inclusive, using June and August rainfall and August temperature as the independent variables. This coefficient was 0.755. It could undoubtedly have been higher if curves had been used, but it was felt that the data were insufficient to justify the use of curves. The regression equation arrived at is given below, A referring to June rainfall, E to August rainfall, and F to August temperature.

$$\text{Yield} = 919.38 - 11.43A - 0.74E - 8.69F$$

Of the three factors involved, A is the most important and E the least. The regressions are all negative, as the curves were, when derived from the longer series. This serves as a partial indication of the predominance of the weevil influence in determining the curves, and therefore encourages the placing of confidence in the curves.

[6] EZEKIEL, M. J. B. A METHOD OF HANDLING CURVILINEAR CORRELATION FOR ANY NUMBER OF VARIABLES. Jour. Amer. Statis. Assoc. 19: 431–453, illus., 1924.

RELATIVE SUSCEPTIBILITY OF SOME RUTACEOUS PLANTS TO ATTACK BY THE CITRUS-SCAB FUNGUS [1]

By JOHN R. WINSTON, *Associate Pathologist*, and JOHN J. BOWMAN and WALTER J. BACH, *Junior Pathologists, Fruit-Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

In a former publication by the senior writer of this paper [2] were listed most of the commercially important citrus plants subject to scab in Florida. The present paper gives a more extensive list indicating the relative susceptibility to the disease as it occurs on its hosts in central and south Florida.

The data presented here are based on: (1) Observations made in commercial citrus nursery and grove plantings during more than seven years, viz, 1916 to 1924; (2) studies of rare forms of undetermined susceptibility closely interplanted with sour orange seedlings severely affected with scab; and (3) inoculation tests made on many rutaceous plants growing on the grounds of the field station of the United States Bureau of Plant Industry at Orlando, Fla. The method used in these inoculation tests has already been described.[3]

Citrus scab is caused by the fungus usually but erroneously referred to as *Cladosporium citri* pro tem Massee. Anna E. Jenkins,[4] assistant mycologist in the Bureau of Plant Industry, United States Department of Agriculture, has published a paper dealing with the morphology and taxonomy of this organism and has described the scab pathogene as *Sphaceloma fawcettii* n. sp.

Observations and inoculations on commercial citrus varieties have been extensive enough to be thoroughly dependable; on the noncommercial varieties and hybrids they have been limited to a small number of plants, and for that reason the general impression of relative susceptibility is given tentatively. Further observations may indicate that these hosts may be affected in a degree different from that reported herein. Many of the rutaceous plants under study are quite small and have not fruited; therefore observations were limited to occurrence of the disease on leaves and tender twigs. However, for those forms that have fruited there seems to be no difference in susceptibility of the fruit as compared with leaves and twigs.

RELATIVE SUSCEPTIBILITY TO SCAB

The results of the inoculations and observations on the relative susceptibility of many rutaceous plants to attack from the scab fungus are given in Table I.[5]

It is interesting to note that, with the exception of *Claucena lansium*, the rutaceous plants attacked by scab belong to the subtribe Citrinae. Members of only four genera, viz, Citropsis, Poncirus, Fortunella, and Citrus are known to be affected by this disease. Poncirus and Fortunella until a few years ago were regarded as members of the Citrus genus. Under conditions in central Florida, *Claucena lansium* is attacked moderately, while *Poncirus trifoliata* is only slightly affected. Only once has scab been observed on Citropsis and likewise only once has it been observed on any Fortunella species, in spite of the fact that for years they have been growing close to scab-infected grapefruit and sour orange trees. This kumquat (Fortunella) infection resulted from artificial inoculation.

At no time has scab been observed on four species of Citrus, viz, *Citrus ichangensis*, *C. medica*, *C. janos*, and *C. webberi*.

Several varities of lemon (*Citrus limonia*) are extremely liable to attack by scab, while the sweet lemon and the dwarf Chinese lemon have thus far been observed to be distinctly less susceptible to severe injury.

The Kusaie and Sour Rangpur lime (*C. aurantifolia*) are severely attacked by scab; the Tahiti lime has been found

[1] Received for publication Sept. 24, 1924; issued August, 1925.

[2] WINSTON, J. R. CITRUS SCAB: ITS CAUSE AND CONTROL. U. S. Dept. Agr. Bul. 1118, 39 p., illus. 1923.

[3] WINSTON, J. R. Op. cit., p. 23.

[4] JENKINS, A. E. THE CITRUS SCAB FUNGUS. Phytopathology 15: 99–104, illus. 1925.

[5] The botanical classification of rutaceous species and hybrids referred to in this paper follows W. T. Swingle in his articles in BAILEY, L. H. THE STANDARD CYCLOPEDIA OF HORTICULTURE. 6 v. New York. 1914. Entry under genus names.

TABLE I.—*Relative susceptibility of some rutaceous plants to citrus scab*

Botanical name	Common or variety name	Range of susceptibility				
		No infection observed	Very rarely attacked	Slightly susceptible	Moderately susceptible	Very susceptible
Family Rutaceae:						
Subfamily Citratae—						
Glycosmis pentaphylla D. C.		×				
Claucena lansium Sk.					×	
Chalcas (Murraea) exotica Mills.		×				
Tribe Citreae—						
Subtribe Aeglinae—						
Feronia limonia Sw.		×				
Feroniella lucida Sw.		×				
Aeglopsis chevalieri Sw.		×				
Aegle mamelos Cor.		×				
Chaetospermum glutinosa Sw.		×				
Balsamocitrus gabonensis Sw.		×				
Subtribe Lavanginae—						
Hesperethusa crenulata Roem.		×				
Triphasia trifolia P. Wilson.		×				
Paramignia monophylla Wight.		×				
Severinia buxifolia Ten.		×				
Subtribe Citrinae—						
Citropsis schweinfurthii S. and K.				×		
Poncirus (Citrus) trifoliata Raf.				×		
Atlantia citroides Pierre.		×				
Fortunella hindsii Sw.		×				
F. margarita Sw.	Nagami kumquat (oval).		×			
F. japonica Sw.	Marumi kumquat (round).	×				
F. crassifolia Sw.	Meiwa kumquat	×				
Citrus ichangensis Sw.		×				
C. medica Linn.	Corsican citron	×				
Do.	Etrog citron	×				
C. limonia Osbeck	Dwarf Chinese lemon				×	
Do.	Kenedy lemon					×
Do.	Lamb lemon					×
Do.	Rough lemon					×
Do.	Sweet lemon				×	
Do.	Villa Franca lemon					×
C. janos Tan.	Kansu orange	×				
C. aurantifolia Sw.	Kusaie lime					×
Do.	Mexican lime	×				
Do.	Persian lime	×				
Do.	Sour Rangpur lime					×
Do.	Tahiti lime			×		
Do.	Thornless lime	×				
Do.	Woglum lime	×				
C. grandis Osbeck	Conner grapefruit				×	
Do.	Davis grapefruit				×	
Do.	Duncan grapefruit				×	
Do.	Foster grapefruit				×	
Do.	Gold Medal grapefruit				×	
Do.	Hall (Silver Cluster) grapefruit.				[a] ×	
Do.	Leonardi grapefruit				×	
Do.	Marsh grapefruit				[b] ×	
Do.	Pink Marsh grapefruit				×	
Do.	McCarty grapefruit				×	
Do.	Pernambuco grapefruit.				×	

[a] More susceptible than most commercial varieties.
[b] Less susceptible than most commercial varieties.

TABLE I.—*Relative susceptibility of some rutaceous plants to citrus scab*—Contd.

Botanical name	Common or variety name	Range of susceptibility				
		No infection observed	Very rarely attacked	Slightly susceptible	Moderately susceptible	Very susceptible
Family Rutaceae—Continued.						
Subfamily Citratae—Continued.						
Tribe Citreae—Continued.						
Subtribe Citrinae—Con.						
C. grandis Osbeck	Royal grapefruit	×				
Do	Triumph grapefruit	×				
Do	Walters grapefruit				×	
Do	Chinese pummelo				×	
Do	Pink pummelo				×	
Do	Sour pummelo				×	
Do	Common shaddock				×	
Do. (?)	Cuban shaddock	×				
C. aurantium Linn.	Bergamot orange	×				
Do	Bitter sweet orange					×
Do	Willow leaf bitter sweet orange				×	
Do	Myrtle leaf orange				×	
Do	Otaheite orange	×				
Do	Sour orange					×
C. sinensis Osbeck	Chamoudi orange	×				
Do	Florida Seedling orange		×			
Do	Enterprise orange	×				
Do	Homosassa orange	×				
Do	Jaffa orange	×				
Do	Lamb orange	×				
Do	Lue orange		×			
Do	Maltese orange		×			
Do	Mediterranean sweet orange		×			
Do	Norris early orange	×				
Do	Parson Brown orange	×				
Do	Pineapple orange		×			
Do	Ruby orange	×				
Do	Surprise orange	×				
Do	Valencia orange	×				
Do	Washington navel orange		×			
C. nobilis Lour.	King orange					×
C. nobilis deliciosa Sw.	Clementine orange				×	
Do	Cleopatra mandarin	×				
Do	Dancy tangerine				×	
Do	Oneco tangerine				×	
Do	Mandarin				×	
C. nobilis unshiu Sw.	Satsuma				×	
C. mitis Blanco	Calamondin					×
C. (Papeda) hystrix D. C.	Pointed leaf papeda				×	
Do	Round leaf papeda	×				
C. webberi Wester		×				
Microcitrus australasica Sw.	Australian finger lime	×				
Subfamily Toddalioideae—Tribe Toddalieae—Subtribe Toddalinae—						
Toddalia lanceolata Lam.		×				

affected with scab on several occasions; while the Persian, which is very similar to the Tahiti, the Mexican, the Thornless, and the Woglum lime, appear to be immune.

Most grapefruit (*Citrus grandis*) varieties are only moderately affected by scab. The Hall appears to be more susceptible than most varieties, while the Marsh seems less likely to be seriously attacked, but this difference is not sufficiently great to justify separate spray schedules for these two varieties. The apparently immune Royal and Triumph varieties are regarded as hybrids of orange and grapefruit. On the other hand, the Leonardi variety, which possesses a number of the distinctive growth features of the Royal and Triumph, and which is considered by some to be an orange and grapefruit hybrid, is about as susceptible as most commercial varities of grapefruit. The pummelos are about as susceptible as most grapefruit. Scab has not been observed on the Cuban shaddock (considered by some to be a hybrid), a fact which increases

its potential value as a rootstock for nursery use, because scab has a marked stunting effect upon very young trees of susceptible varieties.

The Sour and Bittersweet oranges (*Citrus aurantium*) are severely attacked by scab; the varieties Myrtle Leaf and Willow Leaf are moderately attacked, but the disease has not been observed on the Bergamot and Otaheite forms.

The common or sweet Florida orange of commerce (*Citrus sinensis*) is highly resistant to scab. This species is affected so rarely that for all practical purposes it may be considered immune. A trace of scab has been observed a very few times on each of these following varieties—Florida Seedling, Lue, Maltese, Mediterranean Sweet, Pineapple, and Washington Navel. The diagnoses in these cases were verified by cultures from the affected parts. A scab-like blemish of undetermined cause has been observed occasionally on the sweet orange, but microscopic and cultural studies have consistently failed to reveal the scab fungus in the affected tissue.

The kid-glove oranges (*Citrus nobilis*) on the average at least, are as likely to develop scab as the grapefruit. The King variety is very susceptible, the Satsuma is distinctly less severely attacked, while the Cleopatra mandarin appears to be immune. Because of its immunity to scab and because it develops an excellent root system, the Cleopatra mandarin is gaining popularity as a rootstock in Florida.

The calamondin (*Citrus mitis*) is about as severely affected as the King.

The pointed-leaf form of Papeda (*Citrus hystrix*) is also severely attacked by scab, while the round-leaf form has not developed this disease.

Thus it seems that in the several groups susceptible to scab infection there is at least one commercial member on which scab occurs, though *Citrus sinensis* and *Fortunella margarita*, for practical purposes, are immune.

CITRUS HYBRIDS

Observations have been made on a rather large range of citrus hybrids and the degree of infection recorded for them is tentative and unless otherwise stated refers to leaf infections only. Many of these forms most likely will never be grown extensively, and only a few of them are now distributed through the citrus belt of Florida. For these reasons the relative susceptibility to scab can not be determined accurately at this time nor possibly in the near future. Table II gives the impressions gained from inoculations and numerous observations on the degree of scab infection on some citrus hybrids.

The data in Table II show that when both parents of the hybrid are attacked by scab the progeny in general is more likely to be affected by that disease than when only one parent is attacked, and a hybrid resulting from the crossing of two scab-resistant forms is likely to inherit the scab resistance of its parents. With but few exceptions, citrus hybrids are at least as severely attacked as the most susceptible parent. The only hybrid under observation with a scab-susceptible parent that has thus far been scab free is the Faustrimedin, a plant that does not resemble the Calamondin in any of its gross characters. It seems from the data in Table II that liability to scab attack is transmitted from susceptible parents to the first-generation progeny.

CITRUS SCAB OUTBREAKS

Citrus scab is very erratic in its outbreaks. Tender growth is the most highly susceptible. The outbreaks vary not only from season to season, depending on weather conditions, but with individual trees. The variation with different trees seems to be due to the fact that under Florida conditions a citrus tree may start growth as much as two weeks or more before the surrounding ones and even on one side of a tree new growth may sometimes be a week or more slower in starting than on the other. This disease also varies from district to district. The grapefruit and lemon in the Rio Grande Valley are affected by scab much less than in Florida. In Alabama the Satsuma and the Trifoliata orange are much more severely attacked than the same varieties are in Florida. Peltier and Frederich [6] report that in southern Alabama scab has not been observed on the Orangelo, Siamelo, or Siamor. The disease has been observed on these hosts in Florida. The Citrangequat seems to be more severely attacked in Alabama than in Florida.

The difference in degree of scab outbreak on a host in the several States seems more likely to be due to climatic conditions while the host is at a susceptible stage of growth rather than to fluctuating differences in inherent susceptibilities of the hosts or to altered virulence in strains of the pathogene.

[6] PELTIER, G. L., and FREDERICH, W. J. RELATIVE SUSCEPTIBILITY OF CITRUS FRUITS AND HYBRIDS TO CLADOSPORIUM CITRI MASSEE. Jour. Agr. Research 24: 955–959. 1923.

TABLE II.—*Relative susceptibility of citrus hybrids to citrus scab*

BOTH PARENTS SUSCEPTIBLE

Name of hybrid	Female parent	Male parent	C. P. B.[a] office numbers for hybrids	Range of susceptibility			
				No infection observed	Slightly susceptible	Moderately susceptible	Very susceptible
Citranguma	Citrus nobilis unshin Sw. (Satsuma)	Morton citrange	48055				×
Citremon	C. limonia Osbeck (Lisbon lemon)	Poncirus trifoliata Raf. (Trifoliata orange)	46163, 46219, 46356			×	
Citrumelo	C. grandis Osbeck (Bowen grapefruit)	do	4477		×	×	
Clemelo	C. grandis Osbeck (Duncan grapefruit)	Citrus nobilis delicosa Sw. (Clementine)	49851, 49859, 49862, 49866, 49910.			×	×
Do	C. grandis Osbeck (Bowen grapefruit)	do	49026, 49039, 49059			×	×
Do	C. nobilis delicosa Sw. (Clementine)	Citrus grandis Osbeck (Bowen grapefruit)	49933, 49941			×	×
Lemelo	C. grandis Osbeck (Chinese pummelo)	Citrus limonia Osbeck (Myers' lemon)	11045				×
Pomelolo	C. grandis Osbeck (Savage grapefruit)	Citrus grandis Osbeck (Marsh grapefruit)	40829			×	
Rangelo	C. aurantifolia Sw. (Rangpur lime)	Citrus grandis Osbeck (Sour pomelo)	52111			×	×
Satsumelo	C. nobilis unshin Sw. (Satsuma)	Citrus grandis Osbeck (Triumph grapefruit)	7270, 52022			×	
Do	do	Citrus grandis Osbeck (Bowen grapefruit)	52001, 52009, 52010, 52011.			×	×
Siamelo	C. nobilis Sw. (King orange)	do	50506, 50544, 51538, 51541, 51566, 51589, 51594, 51597, 51882, 51894, 51959, 51981, 51990, 52007, 52028, 52030.			×	×
Do	C. grandis Osbeck (Bowen grapefruit)	Citrus nobilis Sw. (King orange)	52013, 40101, 47043, 47044, 47047, 47075, 47286, 52013, 52015.			×	×
Do	C. nobilis Sw. (King orange)	Citrus gradis Sw. (Duncan grapefruit)	44368			×	×
Sopodia	C. grandis Osbeck (Sour pomelo)	Citrus aurantium Linn (Sour orange)	52038			×	
Soporin	C. nobilis Sw. (King orange)	Citrus grandis Osbeck (Sour pomelo)	51289, 51310, 51519, 51524.			×	×
Tangelo	C. grandis Osbeck (Bowen grapefruit)	Citrus nobilis delicosa Sw. (Tangerine)	52016, 52018				×
Tangelo, Sampson	Grapefruit	Dancy Tangerine					×
Tangelo, Sampson seedling.			7140				×
Tangelo, Thornton	Grapefruit	Dancy Tangerine					×
Tangelo, Webber	Second generation of Sampson seedling		L-762, L-765				×
Tangelolo	Citrus grandis Osbeck (Savage grapefruit)	Tangelo	40969				×
Do	do	Sampson tangelo	47206, 47207, 47214, 47220, 47222.				×
Do	C. grandis Osbeck (Bowen grapefruit)	Tangelo	52014				×
Tangelorin	C. nobilis Sw. (King orange)	do	51106, 51115				×

[a] Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, U. S. Department of Agriculture

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TABLE II.—*Relative susceptibility of citrus hybrids to citrus scab*—Continued

Name of hybrid	Female parent	Male parent	C. P. B. office numbers for hybrids	Range of susceptibility: No infection observed	Slightly susceptible	Moderately susceptible	Very susceptible
ONE PARENT SUSCEPTIBLE AND ONE RESISTANT							
Citradia	Poncirus trifoliata Raf. (Trifoliata orange)	Citrus aurantium Linn (Sour orange)	50049			×	
Citrage, Rusk	do	Citrus sinensis Osbeck (Seedling orange)	11540		×	×	
Citrage	Citrus sinensis Osbeck (Washington navel)	Poncirus trifoliata Raf. (Trifoliata orange)	45152, 45169, 45174		×	×	×
Citragequat, Thomasville	Fortunella margarita Sw. (Oval kumquat)	Willits citrange	48010		×		
Faustrimedin	Microcitrus australasica Sw. (Australian finger lime)	Citrus mitis Blanco (Calamondin)	47431	×			
Lemonime	Citrus aurantifolia Sw. (Mexican lime)	Citrus limonia Osbeck (Genoa lemon)	48848			×	
Lemonquat	C. limonia Osbeck (Genoa lemon)	Fortunella margarita Sw. (Oval kumquat)	48770			×	
Limelo	C. aurantifolia Sw. (Mexican lime)	Citrus grandis Osbeck (Sour pummelo)	47123			×	
Do	C. aurantifolia Sw. (Rangpur lime)	do	52111			×	
Orangelo	C. grandis Osbeck (Bowen grapefruit)	Citrus sinensis Osbeck (Parson Brown orange)	40952			×	
Do	do	Citrus sinensis Osbeck (Lamb's summer orange)	49078, 49178			×	
Do	do	Citrus sinensis Osbeck (Ruby blood orange)	47022			×	
Siamor	C. nobilis Sw. (King orange)	do	52029				×
BOTH PARENTS RESISTANT							
Faustrime	Citrus aurantifolia Sw. (Mexican lime)	Microcitrus australasica Sw. (Australian finger lime)	49810, 49823	×			
Limequat	do	Fortunella japonica Sw. (Round kumquat)	48798	×			
PARENTAGE NOT FULLY KNOWN							
Lemon hybrid	Probably Citrus limonia Osbeck (lemon) and Citrus medica Linn. (citron)		S. P. I. No. 23367[b]				×
Microcitrus hybrid	Probably Microcitrus australasica Sw. (Australian finger lime) and Microcitrus australis Sw. (Dooja lime)		7775	×			
Natsumikan	Probably Citrus grandis Osbeck (Pummelo) and Citrus nobilis Sw.		11184, 11337		×		
Tangelo			S. P. I. No. 19610[b]				×
Tangelo ? Wales							×
Temple	Orginal tree, parents unknown						×
Do	Bud progeny						×
Do	Seedling		11159				×

[b] Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, U. S. Department of Agriculture.

SUMMARY

The citrus scab fungus attacks a comparatively narrow range of plants in the family Rutaceae, as indicated by observations and inoculations made on 2 subfamilies, 2 tribes, 4 subtribes, 22 genera, 35 species, 71 varieties, and 47 hybrid combinations. Many citrus forms appear to be immune, while the susceptible ones exhibit all grades of resistance.

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EFFECTS OF CROPS ON THE YIELDS OF SUCCEEDING CROPS IN THE ROTATION, WITH SPECIAL REFERENCE TO TOBACCO [1]

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INTRODUCTION

The fact that continuous culture of any particular crop on the same soil quite commonly results, sooner or later, in decreased yields must have been observed in the earliest days of agriculture. Solution of the problem as to the cause or causes involved, however, has proved to be decidedly difficult. Macaire (*15*),[2] in 1832, remarked that in the broadest sense the rotation of crops is as old as agriculture itself, having come into practice as a matter of necessity, while he further stated that the definite formulation of a theory of crop rotation in the early part of the nineteenth century marked an important advance in the science of agriculture. Two principal theories have been offered in explanation of the unfavorable influence of one plant on another of the same or different species which is frequently observed when the two are grown in association or when the one occupies the soil in advance of the other. The oldest of these theories, involving the idea that a plant may excrete through its roots substances capable of exercising varying degrees of toxicity with respect to itself and other plants, was developed mainly by De Candolle (*3*) in his Physiologie Vegetale, published in 1832. Clements, in a recent monograph (*4, pp. 144–162*), has given a comprehensive review of the fairly extensive literature on the subject of toxic exudates of plants and the rather closely related conception of soil toxins emanating from plant débris, so that for present purposes it will suffice to refer very briefly to these theories.

De Candolle based his theory of toxic root excretions largely on the results obtained by Macaire (*15*). In maintaining well-developed plants in pure water for a time Macaire found that the water acquired toxic properties and contained substances which were regarded as plant excretions. De Candolle considered that plants tend to affect the soil unfavorably for other plants of the same species, genus, or family, and drew a distinction between this sort of unproductivity of the soil and general soil exhaustion affecting all crops and due to lack of plant food. He also stated that dead plant material may be either harmful or beneficial in intermediate stages of decomposition, but is generally beneficial when more fully decomposed. Macaire suggested that the very ancient practice of fallowing or resting the land really involves a rotation of cultivated crops and adventitious vegetation, and that replacement of weeds by useful crops in the rotation is to be regarded as a distinct step in the progress of scientific agriculture. In the light of the results of cropping tests presented in this paper, one needs to be fairly specific both as to crops to be used and conditions under which they are to be grown in putting into effect this seemingly logical suggestion of Macaire.

De Candolle's theory of toxic root excretions soon gave way, at least for a time, to the "plant-food theory" developed by Liebig, according to which soil productivity may be measured primarily in terms of the available supply of plant nutrients. Differences in the effects of crops on the yields of succeeding crops would thus be explainable on the basis of differences in the kinds and quantities of plant nutrients removed from the soil by the different crops. For the greater part of a century this theory has largely dominated field experimentation in soil productivity, though it has as a whole undergone considerable modification during this time. There can be no doubt as to the fact that the supply

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[2] Reference is made by number (italic) to "Literature cited," pp. 1132.

of available nitrogen and mineral nutrients in the soil is one of the important factors in crop yields. On the other hand, inability of investigators to arrive at a satisfactory basis for generalization in the use of fertilizers, after an enormous amount of experimentation, has brought about a realization that there are other factors in soil productivity of fundamental importance. Thus, at the beginning of the present century there was a revival in modified form of the theory of soil toxins wherein these toxins are considered as of vegetable origin but not necessarily consisting of excretions of the living plant.

Among the leaders in the development of the newer theory of soil toxins were Bedford and Pickering in England, and Whitney, Cameron, and Livingston in this country. While Liebig was developing his plant-food theory other investigators were pointing out that Macaire's supposed toxic root excretions might well originate merely from exfoliated root material, and, in fact, De Candolle himself, as already stated, had included consideration of the effects of plant débris in outlining his theory. Bedford and Pickering (*1*) made an extended study of the harmful effects of grass on the growth of fruit trees, and also made observations on many other plants. Whitney and Cameron (*20*) and Livingston, Britton, and Reid (*14*) found that certain unproductive soils as well as their aqueous extracts contained substances toxic to plants, while their toxicity was reduced by addition of certain forms of organic matter, oxidizing agents, and other chemicals, including plant nutrients. Schreiner and his associates (*16*, *17*) isolated from soils several organic compounds which were found to be toxic to plants.

Mention is made also of a recent extension of the theory of soil toxins to include the action of such inorganic toxic substances as soluble salts of aluminum and iron, more particularly as a feature of the soil condition spoken of as acidity. There has been considerable controversy between adherents of the newer theory of soil toxins and those favoring the plant-food theory, and this has brought about a great deal of valuable experimentation. In going over the published data on the subject one can scarcely escape the conclusion that in many instances investigators have gone too far in their interpretations of results obtained in reasoning from the particular to the general, while, on the other hand, some have apparently erred in placing unwarranted restrictions in their interpretations of available data. It appears that further work is needed to reach a final conclusion as to the exact bearing of these two theories on soil productivity.

In recent years plant parasitism has come to be recognized as an important factor in soil productivity. As a rule, parasitic microorganisms are more or less specific in their ability to attack higher plants, so that continuous culture of the same or closely related crops would favor development of parasitic disease and thus reduce crop yields. Many cases of this sort have been brought to light in recent years. Ordinarily the causal organism can be readily recognized, but this is not always the case, and definite exclusion of parasitism as a factor in the unfavorable influence of one crop on another is not always a simple matter. Parasitism alone, however, hardly furnishes a sufficient basis for generalization concerning the effects of crop plants on others growing in association or in rotation. Finally, while it is now known that nonparasitic soil microorganisms may greatly affect soil productivity, this fact does not seem to call for a separate theory of general applicability to explain crop effects.

As to field experimentation dealing specifically with the effects of one crop on another, Daubeny (*6*) in England was the first to undertake an extended study of the subject. Oats, barley, flax, tobacco, potatoes, beans, clover, and other crops were grown on small plots over a period of some ten years in continuous cultures and in rotation. As an average for all cropping combinations, rotation gave better yields than continuous culture for most crops, but in many instances this was not true of individual crop combinations. Daubeny's work has been cited in support of the plant-food theory, but Daubeny himself took a very conservative attitude in the matter, although he found little evidence of harmful root excretions.

Bedford and Pickering found that clover as well as grass may adversely affect the growth of fruit trees, whereas weeds do also but to less extent than grass. It was concluded that the toxic action does not accumulate in the soil and lasts only while the surface crop is growing. Some varieties of apples were affected more than others. In supplementary tests with specially devised pots, various crops were affected in varying degrees by the leachings from other growing plants. Hedrick (*11*) observed that on a rich soil

under irrigation in Utah catch crops of vegetables and small fruits exercised characteristic effects on color of foliage and growth of peach trees. In pot tests with a well-fertilized soil, cover crops of tomatoes, potatoes, oats, rye, rape, mustard, crimson clover, peas, and beans affected young peach trees somewhat differently. Oats and rye were most injurious, while beans and clover were beneficial. In a field test at Gizeh, Egypt, under arid conditions, Fletcher (*7*) grew corn and sesamum in alternating rows, different sections of the rows receiving different quantities of water and fertilizer. The corn greatly retarded the growth of the sesamum, and this effect was not overcome by the watering and fertilizing. Skinner (*19*) made the observation that cabbages did not grow well on a peat soil at Middle River, Calif., which had previously grown sesame.

The Howards (*12*) found that early turning under of a green-manure crop is essential for best results with tobacco, for sufficient time must be allowed for decay of the green manure before the tobacco is transplanted. It is stated that, for tobacco, green-manuring with *Crotolaria juncea* is successful only on light, high-lying, well-drained soils. On heavy or water-logged soils, green manure reduces the yield of tobacco. Stress is laid on the necessity of adequate soil aeration in tobacco culture. Hartwell and associates (*9, 10*) conducted a field experiment in which 16 crops were grown on separate plots for two seasons, while a single crop was grown on all the plots in the third season. All plots were fertilized alike. After the various crops the yields of onions ranged from 13 to 412 bushels per acre; the yields of buckwheat ranged from 4 to 34 bushels; the yields of clover hay ranged from 2.5 to 4.3 tons. When the soil was limed to neutrality, the differences in the effects of the various crops on those which followed were much reduced. The effects of the preceding crops were not proportional to the quantities of plant nutrients removed from the soil. The soil acidity was affected differently by the several crops. According to Sewell (*18*) it is the general observation in the Great Plains area that wheat does not yield as well after Kafir as after *Zea mays* and in a field plot test extending over six years a gain of 3 bushels per acre was obtained with wheat after corn over the yield obtained after Kafir. The unfavorable effect of the Kafir is attributed to its toxic action on wheat.

Tobacco has been an important crop in certain sections for more than three centuries, and by farmers the crop has long been regarded as one which exhausts soils and some have considered it as especially injurious to the crop-producing power of the soil. Cocke (*5, p. 19*) in 1860 wrote: "Tobacco has been literally the besom of destruction, which has swept over this once fertile region [the tidewater region of Virginia], and reduced it to a state too poor to remunerate labor employed in its production." One reason for the development of this view is that a large portion of the tobacco crop has always been grown on light, sandy soils which naturally have a comparatively low crop-producing power. On the other hand, certain of these soils yield the best quality of tobacco, so that the tendency has been to grow tobacco year after year on the same soil. Under these circumstances, the system of farming rather than any peculiarity of the tobacco crop might account for the decline in general productiveness of these tobacco soils.

The early settlers learned that, in general, tobacco grew especially well on virgin soil, and it at once became the practice to constantly clear new land for tobacco. It was soon observed, however, that decline in tobacco yields under continuous culture of the crop is not always due to general exhaustion of the soil. Jones (*13, pp. 36–42*), writing in 1724, states: "When land is tired of tobacco it will bear Indian corn or English wheat, or any other European grain or seed, with wonderful increase." Some types of tobacco are grown on exceedingly rich soils, but, nevertheless, it is not possible to grow on such soils more than one or two satisfactory crops of tobacco in a period of some eight to ten years. In other cases exceedingly heavy applications of manures and fertilizers have failed to restore, for tobacco growing, the productiveness of soils on which the tobacco yield has declined under continuous culture. Finally, the yield of tobacco under continuous culture of the crop does not necessarily decline on all soils. In some sections tobacco has been grown each year on the same soil for more than a half century without decrease in yield.

PURPOSE AND GENERAL PLAN OF THE EXPERIMENTS

As the area of cleared land in the older tobacco-growing sections constantly increased, the practice of resting

basic rotation of a hoed crop with a small-grain and a leguminous soiling crop, not as applied simply to a particular representative of each group to make up a single rotation, but applied under comparable conditions to each of three hoed crops, three small grains and four legumes, with addition of a grass mixture as a nonleguminous soiling crop. Thus each of the three hoed crops is grown in rotation with each of the three small grains and each of the five soiling crops, or in 15 complete rotations. The hoed crops also are grown in continuous culture and alternating among themselves, and in rotation with the small grains without use of soiling crops.

Viewed from a somewhat different angle, this plan embraces a systematic test of the effects of each crop on succeeding crops, except that the effects of the small grains *inter se* are not included. The basic rotation under study somewhat resembles that stated by De Candolle to have been in use in Norfolk, England, a century ago, consisting of a root crop, a winter cereal, and clover. In the tests in which soiling crops are not included, a comparison of four different fertilizer treatments has been made; while, with the soiling crops included, a calcium phosphate and sulphate of potash have been applied uniformly to all plots.

PRELIMINARY STUDIES

SOIL SURVEY OF THE EXPERIMENTAL PLOTS

The effects of crops on others that follow undoubtedly depend largely on soil conditions, and, moreover, for such a large number of plots as are here involved it is not to be expected that uniformity of soil can be had. It is desirable, therefore, to have as full information as practicable concerning the characteristics of the soils used in the tests. Accordingly, at our request, the Bureau of Soils made a survey[3] of all the plots, the results of which are shown in the soil map and the following report by S. W. Phillips:

The farm is located about one-fourth mile south of Upper Marlboro, Md., on a series of flat to undulating terracelike or bench areas rising above the Western Branch of the Patuxent River. The slopes between the higher and lower flats or benches are rather abrupt. The drainage is excellent, owing largely to the porous nature of the subsoil and the underlying substrata. The materials from which the soils have been derived are of marine origin. They contain considerable glauconitic[4] material or greensand "marl," and shells. In a bluff exposure along the north edge of the farm and in a road cut near the entrance to the fair grounds the greensand content is so high that the whole underlying strata has a greenish color. This material is of a sandy clay texture containing a few gravel and some small shells, which in places are cemented together into a loosely consolidated mass. During rains the greensand is washed out from the road cut, covering the roadbed with a green layer.

This region is known as the Atlantic Coastal Plain. The soil-forming materials of the region were transported by water from the older, higher country of the Piedmont and Appalachian regions to the north. They necessarily have undergone changes since washed from their original place of occurrence by processes of abrasion, solution, and decay. They are high in content of quartz particles and, as above stated, contain glauconite, a mineral not generally present in the soil of the great Coastal Plain region.

The soils of the experiment plots have been included in the Collington series, which typically has a brown or yellowish-brown surface with a yellowish-brown, yellow, or greenish-yellow subsoil. The lower subsoil is usually more friable and contains a higher percentage of coarse-grained sand than the upper portion of the subsoil. The amount of the greensand material varies considerably. In places there is so little that the soil would have been mapped as the Sassafras if such areas had been of sufficient size and importance. There is, nevertheless, some greensand in all of the soil. The range of content is from a percentage so low as scarcely to impart a greenish cast to a percentage high enough to give a very decided greenish color. The deep-green material of the exposures referred to represents the parent material, that is, the material from which the overlying soils have been formed through weathering and decay of the greensand beds. There is in these soils, of course, much material not derived from greensand, but rather from the quartzal and other particles associated with the greensand bed. On the slopes outcroppings of gravel are sometimes present.

Three soil types and five type phases[5] are present on the farm. The texture of the soil and subsoil, that is, the relative content of gravel, sands, silt, and clay, defines the soil-type variations in the depth of the surface layers of sand or sandy loam, and slight variations in the character of the subsoil are the factors which here have governed subdivision of portions of the types into subclasses or phases.

The Collington fine sandy loam (No. 1).—This is the most extensive soil on the farm. It is a brown fine sandy loam to loamy fine sand in the surface portion, this passing at about 7 to 10 inches into the subsurface layer of yellowish-brown or brownish-yellow fine sandy loam or loamy fine sand. The subsoil, beginning at about 16 to 22 inches, consists of yellowish-brown to a reddish-yellow sandy clay or silty clay loam. In places the greensand content is so high that the subsoil has a greenish-brown or dark green color. The greensand is present typically principally in the subsoil, but there may be small amounts in the surface soil as well. Below a depth of about 30 inches the content of coarse-grained material is higher and the subsoil accordingly is more porous and open. On the lower bench, near the tobacco barn, the surface runs higher than the average in content of medium sand, approaching here the texture of a sandy loam. The surface soil of Fields I, III, and V of the highest bench contains a little more fine sand and silt than the average of the Collington fine sandy loam. A few patches here of unimportant size are as heavy as a very fine sandy loam. The content of greensand is higher in the nitrogen and potash plots of the lower bench than in the fields of the higher level. This may be due or partly due to a transportation by rain water of greensand from the outcropping deposit on the slope ot the lower level. In parts of Plots IVb, IVc, and IVd the amount of greensand

[3] Survey made by Samuel W. Phillips, under direction of Hugh H. Bennett, inspector, southern division, Soil Survey Investigations, Curtis F. Marbut, in charge, Bureau of Soils.

[4] Glauconite is a hydrous silicate of potassium and iron.

[5] A phase is a minor soil variation of local importance.

is very low, such areas really representing a low-greensand-content phase of the Collington fine sandy loam.

Collington fine sandy loam, shallow phase (No. 15).—In this phase the surface layer of fine sandy loam or loamy fine sand is not so deep as in the typical soil, ranging from 4 to 12 inches in thickness. This soil comprises a few very small patches at the north ends of Plots IVe and IVf and Plots 1 and 2 of Field I and the north end of the lime plots where the underlying sandy clay or silty clay loam is exposed at the surface in some places. None of this phase is found on the lower benches. It is most important in the eastern half of the upper bench.

Collington fine sandy loam, deep phase (No. 10).—This phase includes areas of Collington fine sandy loam in which the surface and subsurface layers extend to a depth of about 22 to 30 inches before the sandy clay subsoil is reached. The soil is a brown fine sandy loam or loamy fine sand, while the subsurface layer is a yellow to yellowish-brown sandy loam to loamy fine sand, rather loose in some places. Most of the special potash plots and parts of the special potash and magnesium plots of the lower bench, as well as fairly large area of all of the fields on the upper bench of the eastern part of the farm, consist of this soil.

Collington fine sandy loam, sandy subsoil phase (No. 8).—In this phase the surface layer is a brown fine sandy loam or heavy fine sandy loam, while the subsurface consists of yellow or yellowish-brown, somewhat compact fine sandy loam. At a depth of about 18 to 24 inches there is a coarser-textured, more open brown sandy loam or yellow loamy sand, which continues with but little or no change to depths of 36 inches or more. This phase is of small total extent, and is restricted in occurrence to the southern part of Field IV.

Collington loamy sand (No. 3).—This soil is a brown medium sand containing some coarse sand and fine gravel. At about 8 to 10 inches yellowish-brown or yellow loamy sand, with considerable greensand material, comes in and continues to depths of 3 feet or more. Some gravel is present, particularly near areas of gravelly sandy loam, and on these slopes where there are gravel outcrops. This type occupies much of the supplementary special nitrogen and the special potash plots; it also occurs in the southwestern corner of the special nitrogen plots on the lower bench and covers about one-third of Field II on the second level. On the higher level it is found only in the tobacco rotation plots.

Collington fine sandy loam, heavy subsoil phase (No. 11).—This phase includes variations in which the lower subsoil at depths of about 32 to 36 inches consists of a yellowish to yellowish-brown sandy clay. In places some small gravel is found, and greensand is present in the subsoil and in lesser amount in the soil. It occurs chiefly in the supplementary special nitrogen and special potash plots, in Field II and in the tobacco rotation plots.

Collington fine sandy loam, gravely subsoil phase (No. 11g).—This phase differs from the typical Collington fine sandy loam in having a subsoil of sandy clay containing a considerable amount of gravel at depths below about 24 inches. Areas of this phase occur in the supplementary special nitrogen plots, in the special potash and magnesium plots on the lower level, and in Fields I, IV, and V, and the lime plots in the higher level.

Collington gravelly sandy loam (No. 9).—This type is a light-brown gravelly sandy loam, which passes into light yellowish-brown sandy loam at depths of about 8 to 10 inches, this, in turn, passing into yellowish-brown friable sandy clay at about 24 inches. The subsoil is gravelly, in places being difficult to bore into with the soil auger. The subsoil contains varying amounts of greensand, the lower part containing the higher percentage. This type is found in the special nitrogen and supplementary special nitrogen and special potash plots, in plot IVd of the tobacco rotation plots, and in sections B and C of Field IV.

TABLE I.—*Chemical analysis [a] of greensand and Collington sandy loam*

	Greensand at Marlboro	Soil [b] 0 to 7 inches	Subsoil [b] 7 to 36 inches	Soil [b]	Subsoil [b] 9 to 36 inches	Subsoil [c] 8 to 36 inches
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Potash (K_2O)	2.565	0.888	0.445	0.910	0.376	0.858
Soda (Na_2O)	.391	.718	2.401	.418	.692	.980
Lime (CaO)	.170	.155	.110	.155	.210	.140
Magnesia (MgO)	.740	.396	.474	.185	.336	.136
Manganese oxide (MnO)		.030		.035	.037	.037
Iron (Fe_2O_3)	16.306	4.011	9.067	3.632	6.248	9.488
Alumina (Al_2O_3)	.130	2.448	4.097	2.856	4.742	4.011
Phosphoric acid (P_2O_5)	.065	.054	.104	.053	.076	.088
Sulphuric acid (SO_3)	.012	.116	.096	.110	.056	.132
Insoluble	74.049					
Moisture	2.130					
Volatile organic matter	1.975					

[a] From Soil Survey of Prince Georges County, Md. U. S. Bureau of Soils.
[b] Sample from Mullikin, Md.
[c] Sample from Oak Grove, Md.

TABLE II.—*Monthly record of rainfall, in inches, on experimental fields for April, May, June, July, August, and September, 1912–1923*

Month	1912	1913 [a]	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923
April	0.62	6.18	[a] 4.04	[a] 1.33	1.63	3.45	6.05	3.91	4.81	2.78	1.10	3.23
May	1.31	6.07	[a] 1.67	2.33	5.82	2.22	2.00	6.66	1.06	5.07	4.60	1.78
June	4.74	1.81	2.68	2.40	6.03	3.00	2.27	2.29	5.72	.77	8.26	2.99
July	5.18	1.54	2.69	3.05	3.39	3.18	2.11	6.64	2.83	3.14	6.74	3.72
August	.68	3.89	2.80	7.40	2.97	1.46	5.66	4.30	4.30	2.88	3.82	2.86
September	6.13	3.35	.16	.45	2.02	1.77	1.99	1.69	3.32	1.82	.63	2.53
Total	18.66	22.84	14.04	16.96	21.86	15.08	20.08	25.49	22.04	16.46	25.15	17.11

[a] Data for 1913 and for April and May, 1914, and April, 1915, are those of the Weather Bureau for Cheltenham, Md., about 5 miles distant.

RAINFALL

It is of considerable interest to inquire as to what extent weather conditions, especially rainfall, may modify the effects of a crop on succeeding crops. The available records of rainfall on the experimental fields during the spring and summer months for the period of the experiments are given in Table II.

SPECIAL CROPPING TESTS WITH TOBACCO

The plots used in these tests are one-fourth acre in size. The numbers of plots and treatments for each cropping test are as follows:

IV *a, b, c:* Tobacco, wheat, and red clover in a three-year rotation; tobacco fertilized.
V *a, b:* Tobacco, wheat, and crimson clover in a two-year rotation; tobacco fertilized.
VI *a:* Tobacco each year, crimson clover as a cover crop; no fertilizer used.
VI *b:* Tobacco each year, crimson clover as a cover crop; tobacco fertilized.
VI *c:* Tobacco each year, hairy vetch as a cover crop; tobacco fertilized.
VI *d:* Tobacco each year, rye as a cover crop; tobacco fertilized.
VI *e:* Tobacco each year, no cover crop; tobacco fertilized.
VII *a, b:* Tobacco, wheat, and cowpeas in a two-year rotation; tobacco fertilized.
VII *c, d:* Tobacco, wheat, and cowpeas, in a two-year rotation; no fertilizer used.

The location and arrangement of these plots and the general character of the soil are shown in the soil map, the plots being numbered there as above. (Some of the plots included in the survey relate to experiments not involved in the present discussion.) All of the tests were begun in 1912, except those on Plots VI *d* and VI *e*, which were begun in 1916, a period of four years having been allowed to fully establish the legume features of the tests. In Series IV, V, and VII, tobacco was grown on Plot *a* in 1912, Plot *b* in 1913, and so on, in regular sequence. In 1922 plots were added immediately south of Series V *a, b* for the purpose of growing tobacco every second and third year, respectively, in rotation with weeds. Fertilizer has been applied each year to the tobacco crop, but none has been used on the wheat or soil-improving crops. For the years 1912 to 1915, inclusive, the fertilizer consisted of 100 pounds nitrate of soda, 500 pounds 14 per cent acid phosphate and 100 pounds 50 per cent sulphate of potash per acre. Beginning with 1916 the application of nitrate of soda was reduced to 60 pounds per acre but no change was made in the quantities of acid phosphate and sulphate of potash used. In the years 1912, 1913, and 1914 air-slaked burned lime was applied at the rate of 10 bushels per acre to the plots in each series at the time of turning under the legumes in preparation for tobacco. No lime was used in 1915. Beginning with 1916 ground limestone has been applied in all cases after tobacco, prior to seeding wheat. Each plot in Series IV and VI has received 1,000 pounds per acre once in each three-year period, and the plots in Series V and VII have received 650 pounds once in each two-year period. All legumes have been plowed under, except that one crop of hay has been cut each year from the first-year red clover.

In general, excellent stands and good growth of red clover have been obtained with but little winter killing (pl. 1, A). Crimson clover grown in the two-year rotation with tobacco and wheat has usually made excellent growth when it has escaped winter killing. Rather frequently, however, it has suffered severely from cold, and, altogether, has not been a dependable soil-improving crop. In recent years the crimson clover has been damaged considerably by stem-rot disease. With continuous tobacco culture, with fertilizer, crimson clover has been somewhat less successful than in the two-year rotation for the reason that the tobacco crop comes off too late to allow seeding of the clover at the proper time. Where no fertilizer is applied to the tobacco, crimson clover has been almost a complete failure. Hairy vetch has proved to be a thoroughly dependable winter cover crop, and there has been no winter killing of consequence in spite of the fact that it has been necessary to seed it very late in the fall (pl. 1, B). The vetch has furnished large quantities of organic matter for the soil. The cowpeas have always made good growth where fertilizer was applied to the tobacco (pl. 2, A), but the growth has been much less where no fertilizer was used. Data have been obtained on the yields of wheat in these tests but will not be considered in this discussion.

The yields and gross value of tobacco produced under the several systems of cropping are summarized in Table III. The values of the crops from the different plots are based on the judgment of local tobacco buyers.

A.—Excellent stand and good growth of red clover in a 3-year rotation of tobacco, wheat, and red clover. In comparison with other soiling crops, red clover has given best results with tobacco, but it is possible that this is not due so much to the specifically favorable effect of the clover as to the fact that the cropping system virtually involves resting the land for two years prior to transplanting the tobacco crop. (Compare pl. 4, B)

B.—Growth of hairy vetch as a catch crop in continuous tobacco culture. Hairy vetch as a winter cover crop has made excellent growth each year with almost no winter killing. In the early years of the test, vetch, as a soiling crop, gave large yields of tobacco of good quality, but subsequently the yield has been very variable, with a tendency to decline, especially in wet years

A.—Growth of cowpeas in a two-year rotation of tobacco, wheat, and cowpeas. The growth of cowpeas has been large and the crop has added large quantities of nitrogen to the soil. This legume has given somewhat better results with tobacco than have most other legumes tested. None of the legumes, however, has given anything like as large average increases in yield of tobacco as would be expected on the basis of the nitrogen added to the soil

B.—Tobacco in rotation with rye and soiling crops on Field IV. Plot in center shows the slow and very uneven growth after soy beans. Plot to right shows the marked depressing effect of grass as a cover crop. Note the much larger and more uniform growth of tobacco following cowpeas in plot at extreme left

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TABLE III.—*Yields and gross values of tobacco in special cropping tests, 1912–1923*

YIELDS OF TOBACCO LEAF, POUNDS PER ACRE

Plot series and cropping system	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	Average 1912–1923	Average 1916–1923
IV. Tobacco, wheat, red clover	584	884	1,284	1,080	1,200	1,060	1,176	1,264	1,354	1,312	741	1,217	1,096	1,166
V. Tobacco, wheat, crimson clover	416	1,116	1,212	972	1,356	1,108	768	1,266	672	1,022	437	1,280	969	989
VI. *a*. Tobacco, crimson clover, no fertilizer	570	978	1,404	696	676	640	752	342	380	464	105	425	619	473
VI. *b*. Tobacco, crimson clover, with fertilizer	952	1,108	1,568	924	1,384	1,114	882	833	748	912	273	990	974	892
VI. *c*. Tobacco, vetch	916	1,296	1,692	908	1,283	1,604	1,191	730	812	1,152	590	1,560	1,145	1,115
VI. *d*. Tobacco, rye	----	----	----	----	948	834	1,058	667	534	684	467	876	----	759
VI. *e*. Tobacco, fallow	----	----	----	----	824	896	938	1,013	1,002	984	655	904	----	902
VII. *a, b*. Tobacco, wheat, cowpeas, with fertilizer	816	1,304	1,664	920	1,456	1,268	1,124	970	1,288	974	672	1,332	1,149	1,136
VII. *c, d* Tobacco, wheat, cowpeas, no fertilizer	744	896	1,348	756	840	709	960	268	764	604	377	738	750	658

GROSS VALUES OF TOBACCO, DOLLARS PER ACRE

IV. Tobacco, wheat, red clover	58	68	131	163	242	214	378	597	444	374	172	339	265	345
V. Tobacco, wheat crimson clover	42	94	109	161	274	323	229	583	186	234	48	339	219	277
VI. *a*. Tobacco, crimson, clover, no fertilizer	50	74	131	79	107	120	159	52	30	37	3	18	72	66
VI. *b*. Tobacco, crimson, clover, with fertilizer	97	94	190	108	273	323	260	334	182	186	27	261	195	231
VI. *c*. Tobacco, vetch	94	108	198	144	285	430	392	301	254	248	85	371	243	296
VI. *d*. Tobacco, rye	----	----	----	----	178	187	316	287	129	99	68	311	----	197
VI. Tobacco, fallow	----	----	----	----	155	204	266	425	272	312	170	330	----	267
VII. *a, b*. Tobacco, wheat, cowpeas, with fertilizer	94	107	203	151	300	340	351	140	266	217	98	423	224	267
VII. *c, d*. Tobacco, wheat, cowpeas, no fertilizer	64	66	132	83	141	162	263	28	53	62	33	153	103	112

DISCUSSION OF RESULTS

In analyzing the results of the tests it will be profitable to consider the averages for the period covered, the yearly variations, and the general trend under the several treatments, the latter being more readily presented in graphic form. For the whole period of the tests, 1912 to 1923, inclusive, where fertilizer was used, an average yield of about 1,150 pounds of tobacco per acre has been obtained in continuous culture with vetch as a cover crop and in a two-year rotation of tobacco and wheat with cowpeas as a soiling crop. For the same period a yield of about 1,100 pounds of tobacco leaf has been obtained in the three-year rotation of tobacco, wheat, and red clover. The last-named rotation has given the best quality of cured leaf. A yield of somewhat less than 1,000 pounds has been obtained both in continuous tobacco culture with crimson clover as a cover crop and in a two-year rotation of tobacco and wheat with crimson clover as a soiling crop. The latter, however, has given a somewhat better quality of leaf. In continuous culture with crimson clover as a cover crop, and in the two-year rotation with crimson clover and cowpeas as a soil-improving crop, omission of the fertilizer has greatly reduced the yield and value of the tobacco (pl. 3, A). Under these conditions the tobacco after cowpeas and the cowpeas themselves have shown well-defined symptoms of potash hunger, and leaf spot resembling wildfire and black fire of tobacco has been decidedly more prevalent than where fertilizer has been applied.

For the period 1916 to 1923, inclusive, continuous culture of tobacco with fertilizer, but without use of any cover crop, has given a yield of 900 pounds per acre and the product has been of good quality. With rye used as a cover crop there was an unexpected lowering of the average yield of tobacco and the value of the crop was lowered to an even greater degree. The use of crismon clover and cowpeas as soiling crops, without addition of commercial fertilizer, has greatly reduced the yield and value of the tobacco as compared with a tobacco crop every year with fertilizer but without soiling crop. The three-year rotation of tobacco, wheat, and red clover has given best yields and best quality of

A.—Continuous tobacco culture, with crimson clover as a winter cover crop. On plot at right no fertilizer is applied, while tobacco on plot at left is fertilized every year. The plots have been limed uniformly

B.—Tobacco on rested land, 1923. The soil has not been under cultivation for more than 10 years. Photographed August 6. Note the more advanced and uniform growth as compared with the tobacco of the same age shown in A

tobacco. Cowpeas in the two-year rotation give an increase of something more than 200 pounds over continuous tobacco culture without cover crop but there is no increase in value of the product. Vetch as a cover crop gives about the same gain in yield as the cowpeas in the two-year rotation and also gives some increase in value of the tobacco. Of the several legumes, crimson clover continues to give the lowest yields, while as a cover crop in continuous tobacco culture it has been less effective in maintaining yields than in the two-year rotation which includes wheat. Comparing the period 1916 to 1923, inclusive, with the period 1912 to 1923, inclusive, it will be observed that the three-year rotation which includes red clover is the only cropping system showing a gain in yield of tobacco, all others showing no change or a decline.

An outstanding feature of the results with vetch as a cover crop is the extremely wide range in yield of tobacco from year to year. Similar, though somewhat smaller, fluctuations in yield are obtained from other legumes in the rotation and from rye as a cover crop. By way of contrast, the yields in the red-clover rotation and on the fallow plot are relatively constant, except in 1922, when poor yields were obtained on all plots. These relationships are more clearly shown in Figures 1, 2, and 3. In some years vetch as a cover crop has given very large increases in yield while in other years the yield has been actually depressed. Evidently the effect of the vetch is dependent largely on weather conditions. By comparing the yields of tobacco from year to year with the rainfall during the summer months (Table II), it will be found that the large increases in yield of tobacco usually occur in relatively dry seasons and the poor yields usually in wet seasons.

This relationship between the effect of the cover crop and the rainfall, which seems to hold also for other cover crops, is important. It has often been suggested that turning under large quantities of green manures may cut off the normal upward capillary movement of moisture in the soil. This factor could hardly be of significance in the present case, since the unfavorable action of the vetch occurs in seasons of abundant rainfall. Again, cowpeas in the rotation have much the same effect as vetch, and since the cowpeas are grown in the precedng year they would certainly decay sufficiently before the tobacco crop is transplanted to obviate interference with movement of soil moisture. It is plain,

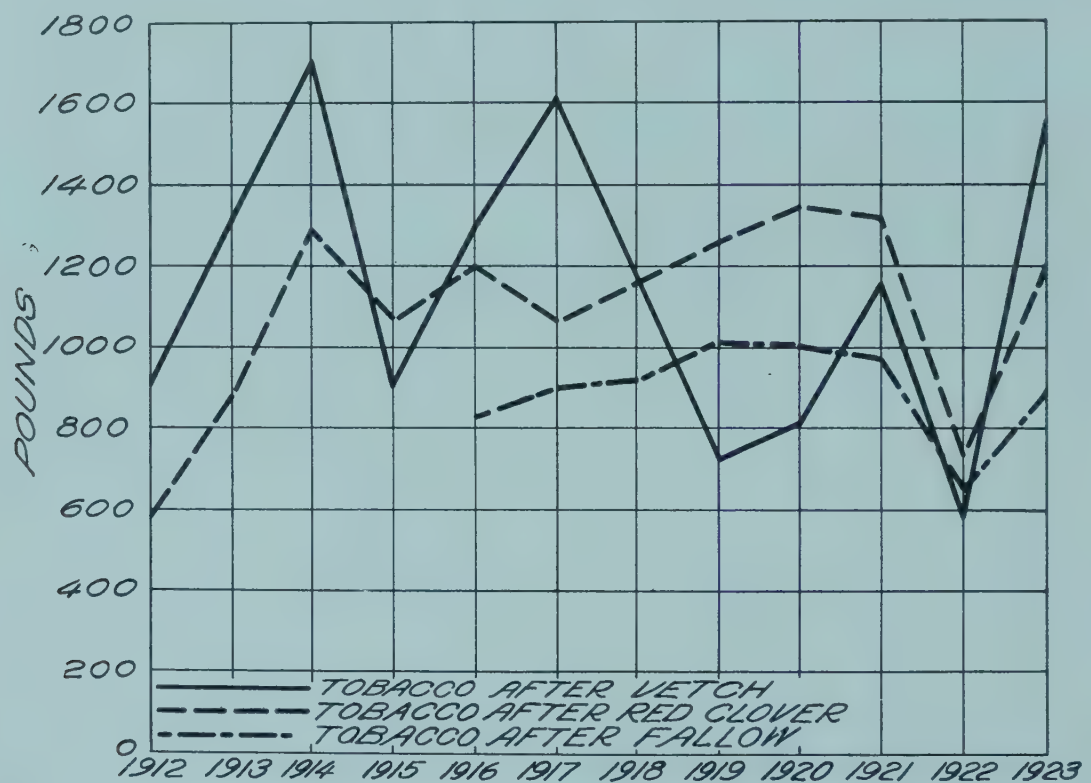

FIG. 1.—Annual yields of tobacco, 1912-1923, inclusive: (1) in continuous culture, with hairy vetch as a cover crop; (2) in a 3-year rotation of tobacco, wheat, and red clover; (3) in continuous culture, with no cover crop. Note the extremely wide fluctuation in yield from year to year and the downward trend in yield after vetch, in contrast with the relatively stabilized yield after clover and with no cover crop and the upward trend in yield after the red clover

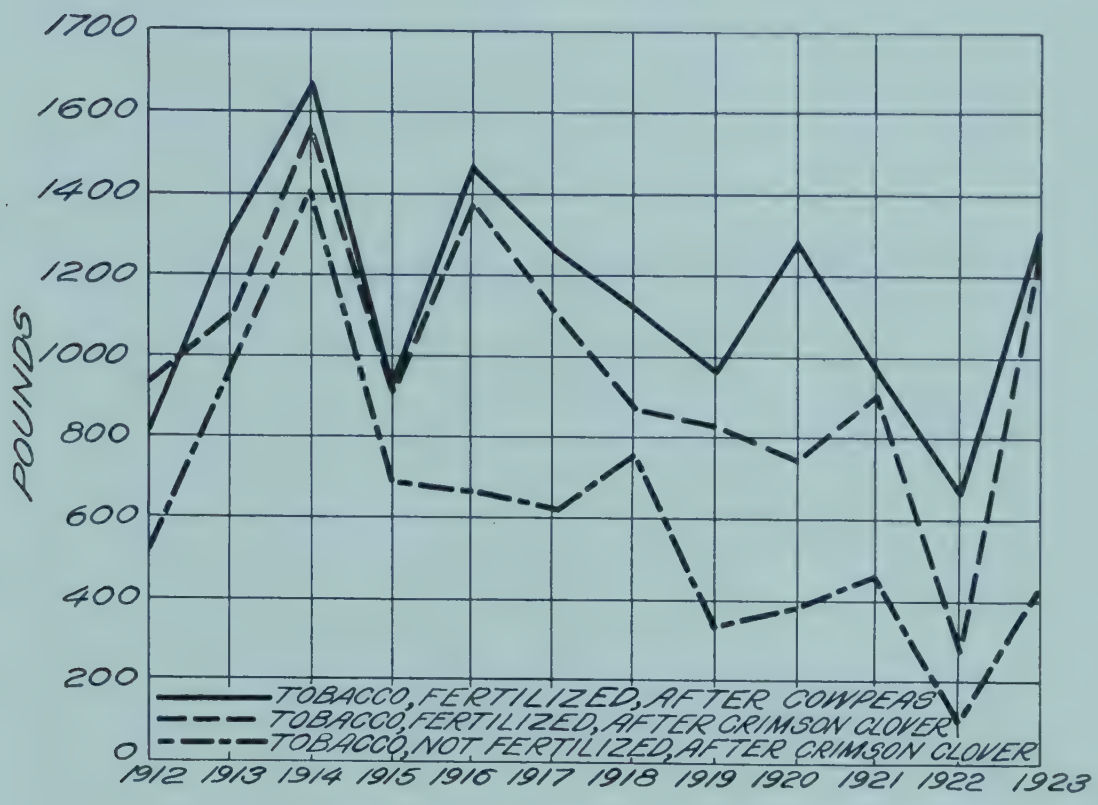

FIG. 2.—Annual yields of tobacco, 1912–1923, inclusive: (1) In a two-year rotation of tobacco, wheat, and cowpeas (turned under), tobacco crop fertilized; (2) in continuous culture, with crimson clover as a cover crop, tobacco fertilized; (3) in continuous culture, with crimson clover as a cover crop, tobacco not fertilized. In all cases the general trend in yield is downward, the initial gains from legumes not being maintained. Omission of fertilizer for tobacco, even when legumes are grown, soon leads to practical crop failure

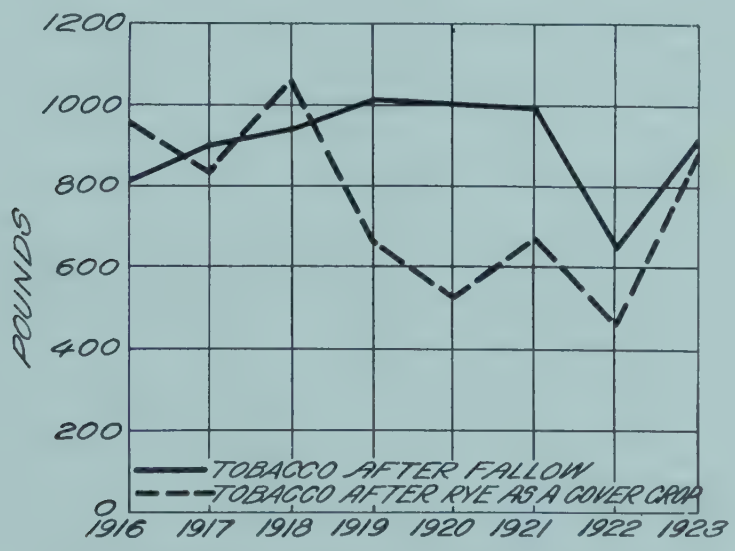

FIG. 3.—Annual yields of tobacco in continuous culture, 1916–1923, inclusive, without use of a cover crop and with rye as a cover crop. At the beginning and in certain subsequent years, the yields after rye were about the same as without cover crop. In relatively wet seasons, however, the yield is markedly depressed by the rye cover crop

also, that the depressing effect of rye on the yield of tobacco is dependent largely on seasonal conditions. With tobacco, an inadequate supply of nitrogen is usually recognizable by the light-green or yellowish-green color of the leaf. After both rye and the leguminous cover crops, the tobacco frequently shows an abnormally dark-green color. A sample of green leaves taken in 1923 from tobacco plants on the vetch plot which had made poor growth showed a nitrogen content of 4.98 per cent in the leaf as against 3.84 per cent in the leaves from large plants on the same plot. Evidently there was no shortage of nitrogen, for usually the nitrogen content of Maryland tobacco does not exceed 2.5 per cent. On the other hand, it is obvious that there could be no excess of nitrogen on the rye plot. The excellent crops of red clover which have been obtained show that the rate of liming has been adequate for ordinary requirements, while anything like excessive liming has been carefully avoided.

The data presented graphically in Figures 1, 2, and 3 indicate for the period of the tests a downward trend in yields from use of rye, crimson clover, vetch, and cowpeas, as soil-improving crops. While the time covered is rather short, it seems unlikely that there will be any permanent return to the relatively high level of yields of the early years. During this same period there has been an upward trend in yields in the 3-year rotations with wheat and red clover, and thus far there has been no significant change in the results where no soiling crop is employed. Whether red clover, as against the other soiling crops used, has some specifically favorable effect on the tobacco crop or the improvement in growth of tobacco is due to virtual resting of the land for two years will require further study. The downward trend in yield where legumes are used but fertilizer omitted has been very marked. In 1922 a series of plots were located immediately south of the older series which provide for 2 and 3 year rotations of tobacco and weeds. The land had not been cropped for many years, and the results with tobacco for 1922 and 1923 really represent tobacco crops on land which had been rested for more than 10 years. The average yields per acre from duplicate plots were 880 pounds in 1922, and 1,332 pounds in 1923, and the corresponding crop values were $251 and $524. In the wet year of 1922 the yield on the rested land was not equaled by that on any of the older cropping plots, and in the generally favorable year of 1923 the yield was exceeded only by that on the vetch plot. In both years the quality of the tobacco on the rested land was decidedly superior to that on any of the older plots.

The reduced yield and value of the tobacco crop which so often occur where soiling crops are used are due primarily to the very uneven growth of the tobacco plants, as shown in Plate 4, A. It frequently happens that many of the stunted plants, after a long delay, make approximately normal growth very late in the season, but under these conditions the quality of the product is almost invariably poor. The even growth on fallow or rested land is shown in Plate 4, B. It is difficult to definitely eliminate parasitism as a possible factor in these results, but it is apparent that any causal parasite must be capable of attacking a wide range of plants, while, on the other hand, it would be necessary to assume a decided selective action as between, for example, red clover and crimson clover. It is to be kept in mind, also, that the soil-improving crops themselves have shown no evidence of injury from disease or decrease in growth during the progress of the tests, except that crimson clover has been injured by the stem-rot disease. Associated with reduced growth of the tobacco, there is reduction in root development and the root system as a whole has a yellowish or brownish color. It is interesting to note that at times the tobacco plants on the rye plot wilt during the middle of the day, whereas there is no indication of wilting on the fallow plot immediately adjoining. Little or no Thielavia root-rot has been found on any of the plots.

From a practical standpoint it seems clear that application of intensive methods which include use of soil-improving crops is not likely to prove altogether successful in tobacco culture, at least under the conditions of these tests. Where land values or other factors increase the necessity for intensive methods, apparently the most promising cropping system is a rotation of at least three years, which includes wheat and red clover. It is hardly to be expected, however, that the results obtained in the present tests will hold true on all tobacco soils; and, in fact, the crop effects here in question seem to be peculiarly dependent both on weather and on soil conditions. It seems fair to assume, moreover, that under proper conditions occasional and cautious use of legumes may give good

A.—Tobacco in continuous culture, with rye as a cover crop. Note the uneven and greatly reduced growth as compared with the crop on adjoining plot. (See B)

B.—Tobacco in continuous culture, without cover crop. Note the uniform growth over the plot as a whole. This plot adjoins that shown in A

results even on the soils used in the present tests. But no system of active cropping seems to give such uniformly good results as resting the land, thus allowing it to be occupied for a time by adventitious vegetation (pl. 3, B).

FIELD STUDY OF THE COMPARATIVE EFFECTS OF TOBACCO AND OTHER CROPS ON THE YIELDS OF SUCCEEDING CROPS

In these tests tobacco has been compared, in all instances, with early potatoes and corn. Each of these crops has been grown (1) in continuous culture and in alternation with each of the other two; (2) in rotation with each of the small grains, wheat, oats, and rye; and (3) in rotation with each of the small grains and each of two winter legumes, two summer legumes, and a grass mixture. Except where the soiling crops are used, four different fertilizer treatments have been applied in each system of cropping. To carry out these tests has required 5 separate fields and a total of 261 plots. The second feature of the tests was begun in 1914, but the entire plan was not in full operation till 1916. The results to be presented at this time are more or less of a preliminary nature and are subject to some modification as the work progresses. For this reason only summarized tabulations of yields will be given, leaving for future publication a more complete presentation of the detailed results for each year.

The general plan in conducting the tests has been to plant the three hoed crops—tobacco, potatoes, and corn—in adjoining, parallel strips of suitable size, the total area embraced in the three strips being designated as a "field." The three strips embraced in a field are called "sections," designated by the letters A, B, and C. In the second year three crops, consisting of the same three hoed crops or three small grains, as the case may be, are planted crosswise the original strips or sections and the strips occupied by the second-year crops, which run at right angles to the sections, are called "divisions." These divisions are distinguished by the numbers 1, 2, and 3. This of course provides for each of the first-year crops to be followed by each of the second-year crops. Thus, each of the three sections of the field occupied by a first-year crop is crossed by each of the divisions occupied by a second-year crop, and vice versa. The effect is to divide the field into nine "cropping units,' each of which embraces a complete 2-year rotation of a first-year and a second-year crop. The location of these cropping units on the field, as well as the cropping system applied to them, are readily identified by combining the section and division designations, as, for example, C 1, A 2.

FERTILIZER TREATMENTS

By making the cropping units of sufficient size they may be subdivided into as many plots as desired for fertilizer tests or for adding legumes to the rotations, both of which features have been made a part of these tests. In this way it becomes possible to compare crop effects with fertilizer effects and, moreover, the plan may be made to furnish a comprehensive test of the fertilizer needs of the soil. In the present tests only four different fertilizer treatments have been used, involving omission of nitrogen, phosphoric acid, and potash, respectively, in comparison with a standard rate of application of the three elements. High-grade dried blood has been used as a source of nitrogen because it supplies only small percentages of phosphorus, potassium, magnesium, sulphur, or other plant-food elements. A chemically precipitated dicalcic phosphate has furnished phosphoric acid, and high-grade sulphate of potash has been used as a source of potash. The standard rates of application per acre, arbitrarily selected, were 20 pounds each of nitrogen and potash and 40 pounds of available phosphoric acid. For convenience in indicating the different fertilizer treatments, the letters N, P, and K are used to designate applications of 20 pounds nitrogen, 40 pounds phosphoric acid, and 20 pounds potash per acre, respectively. The standard rates of application per acre with the corresponding symbols, therefore, are as follows:

1.	20 pounds nitrogen 40 pounds phosphoric acid	N+P
2.	20 pounds nitrogen 20 pounds potash	N+K
3.	40 pounds phosphoric acid 20 pounds potash	P+K
4.	20 pounds nitrogen 40 pounds phosphoric acid 20 pounds potash	N+P+K

None of the fertilizer materials used furnish important quantities of plant nutrients other than the three in question; except that the sulphate of potash supplies approximately 20 pounds per acre of sulphur trioxide and the dicalcic phosphate supplies about 25 pounds of calcium oxide. To avoid any possible shortage of calcium in the soil, and to at least partially overcome any soil acidity, a ton per acre of shell lime-

stone was applied to all plots in 1918 or 1919, and in 1922 a mixture of 2 tons calcite limestone and 1,000 pounds dolomitic limestone containing upwards of 20 per cent magnesium carbonate was applied. Beginning with 1922 sufficient land plaster to furnish the equivalent of the SO_3 content of the sulphate of potash has been applied to the plots which receive no potash. By increasing the size of the cropping units the number of fertilizer treatments may be increased. In later experiments, undertaken in other tobacco-growing sections, plots have been added to include a lime test and the effects of doubling the normal rate of the complete application and of omitting all fertilizer. The size of the present fertilizer plots is approximately one-fortieth acre.

the indicator crops on the control plots may be somewhat affected by the crops of neighboring plots. For these reasons the control plots have not given uniformly satisfactory evidence of relative productiveness in all cases, but for the most part the data are reasonably consistent. In the years 1916 and 1917 the complete fertilizer was applied at the normal rate to the cropping control plots on those fields which were occupied by the hoed crops, but no fertilizer has since been applied to the control plots.

TOBACCO, POTATOES, AND CORN IN CONTINUOUS CULTURE AND IN ROTATION

This test, which was begun in 1916, requires only a single field. This is designated as Field I. The location

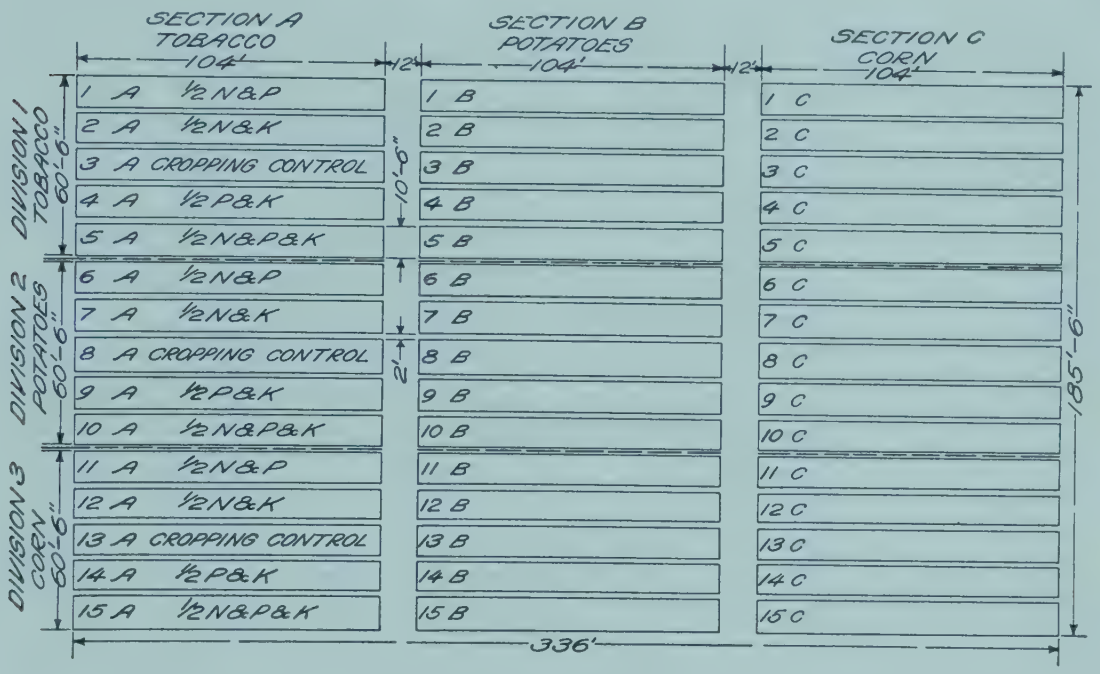

FIG. 4.—Plan of Field I, showing the dimensions of plots and intervening spaces, the fertilizer treatments, and the cropping system

CONTROL PLOTS

In order that information may be had as to the comparative productiveness of different parts of the experimental fields, each cropping unit contains a "cropping control plot," which constitutes the middle plot of the unit. These control plots, of course, are cropped uniformly, tobacco and wheat having been used thus far as indicator crops, though it is now proposed to substitute corn for tobacco. If sufficient land were available, two control plots in each cropping unit would be desirable. It has been observed that different indicator crops may give different results as to relative productiveness of different plots. Also, it appears that under the conditions of the tests

and general plan of the field and the characteristics of the soil are shown on the soil map and accompanying report. The dimensions of the plots and intervening spaces, the fertilizer treatments and the cropping plan are shown in Figure 4.

In 1916 tobacco was grown on Section A, early potatoes on Section B, and corn on Section C. In 1917 tobacco was grown on Division I extending across the upper third of the three sections, potatoes were similarly grown on Division 2, and corn on Division 3. In 1918 the three crops were again grown on their respective sections, while in 1919 the boundaries of the crops again followed the boundaries of the divisions, and so on. It will be observed that tobacco is grown

every year on the block or cropping unit embraced in the first five plots of Section A (upper lefthand corner of fig. 4), this cropping unit being designated as A 1. Tobacco is grown in rotation with potatoes on Plots 6 to 10, inclusive, of Section A, and Plots 1 to 5, inclusive, of Section B, the tobacco crop occupying the first-named plots in even years and the last-named plots in odd years. Tobacco is grown in rotation with corn on the cropping units embracing the last five plots of Section A and the first five plots of Section C. Similarly, potatoes are grown every year on the central block of plots in Section B, and corn is grown every year on the cropping unit embracing Plots 11 to 15, inclusive, of Section C. Potatoes and corn are grown in rotation on the third division of Section B, embracing Plots 11 to 15, and on the second division of Section C, consisting of Plots 6 to 10.

The cropping control plots throughout the field (Plots 3, 8, and 13) are planted to tobacco every year The results on the control plots are summarized in Table IV. The plots are arranged in the table to accord with their actual position in the field, as shown in Figure 4. It appears that the productiveness of the field decreases somewhat from north to south and from west to east.

TABLE IV.—*Comparative productiveness of the cropping units of Field I as indicated by the average yields of tobacco on the control plots for the period 1916–1922, inclusive*

Location of plot	Leaf, average yields per acre			Stalks, average yields per acre		
	Plot 13	Plot 8	Plot 3	Plot 13	Plot 8	Plot 3
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
Section A	987	955	886	603	562	492
Section B	997	921	810	577	492	440
Section C	823	738	822	462	393	458

In 1916 the fertilizer materials were applied to the various plots at the standard rates, but in subsequent years only half the normal rates has been applied, as indicated in Figure 4. The Maryland Broadleaf type of tobacco has been used in these cropping tests as well as in those described later. The McCormick variety of potatoes was used in 1916, and in subsequent years the Irish Cobbler variety has been grown. The Boone County White variety of corn has been used in all the cropping tests of this group. Special effort has been made to insure satisfactory stands of the three crops on all plots. In practically all cases nearly perfect stands of tobacco have been obtained, although in some instances not all plants made sufficient growth to justify harvesting. In 1919 the number of plants actually harvested constituted about 92 per cent of a perfect stand where tobacco followed tobacco, 92 per cent where tobacco followed potatoes, and 90 per cent where tobacco followed corn. Similarly, where tobacco followed tobacco in 1920, 92 per cent of a stand was harvested, and in 1921, 80 per cent of a stand of tobacco was harvested after tobacco, 87 per cent after potatoes, and 90 per cent after corn. In all other cases the stand of tobacco harvested was 95 per cent or better. Practically perfect stands of potatoes were obtained in all instances. The same is true of corn, except that in 1917 the imature ears were damaged approximately 10 per cent by birds where corn followed corn, and in 1921 the stand on the same plots was about 91 per cent.

In comparing the three crops—tobacco, potatoes, and corn—it will be apparent that the valuable portions are, respectively, the leaf, tubers, and grain or seed, representative of three different features of plant growth. In the case of tobacco and corn, approximately the entire portion of the plant above ground is harvested, but this is not practicable for potatoes. Since no crop effects could be produced until the second year of the test, the crop yields of 1916 are not considered in this discussion. The average yields for the years 1917 to 1922, inclusive, under the different cropping combinations and different fertilizer treatments, are summarized in Table V. The tobacco crop of 1919 is not included in the averages because the buds of the young plants were severely injured by flea beetles and the results are not considered reliable.

The average yields of tobacco on the control plots in continuous culture, in rotation with potatoes, and in rotation with corn, are, respectively, 886,

TABLE V.—*Summary of results with tobacco, potatoes, and corn on Field I for the years 1916–1922, inclusive, showing the comparative effects of each crop on yields of succeeding crops of tobacco, potatoes, and corn under different fertilizer treatments*

Fertilizer treatment	Yield of tobacco leaf				Yield of potatoes				Yield of corn			
	After tobacco	After potatoes	After corn	Average	After tobacco	After potatoes	After corn	Average	After tobacco	After potatoes	After corn	Average
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*
½(N+P)	825	1,006	868	900	64.1	54.8	37.8	52.2	49.1	50.2	33.8	44.3
½(N+K)	804	923	853	862	60.3	48.9	37.8	49	53.7	49.5	41.5	48.2
½(P+K)	904	937	960	933	59.1	48.3	34	47.1	49.6	46.5	42.3	46.1
½(N+P+K)	1,099	1,118	1,196	1,138	76.1	57.4	43.1	58.9	53.1	51.8	48.1	51
Average	908	996	969	958	64.9	52.4	38.2	51.8	51.4	49.5	41.4	47.4

Fertilizer treatment	Yield of tobacco stalks				Yield of corn stover			
	After tobacco	After potatoes	After corn	Average	After tobacco	After potatoes	After corn	Average
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
½(N+P)	428	574	528	510	3,282	3,290	2,757	3,110
½(N+K)	376	486	448	437	3,283	3,317	2,903	3,168
½(P+K)	492	556	568	539	3,437	3,160	2,913	3,170
½(N+P+K)	658	742	768	723	3,560	3,613	3,302	3,492
Average	489	590	578	552	3,391	3,345	2,969	3,235

883, and 905 pounds, indicating close agreement in productivity of the plots involved. It appears, therefore, that taking the average of all fertilizer treatments, continuous tobacco culture gives somewhat reduced yields as compared with tobacco after potatoes and after corn. As a matter of fact, there has been a progressive decrease in yield of tobacco under the continuous culture system, while good yields were obtained in the early years, so that the average result does not fully portray the situation. It is surprising that there is no substantial difference in yields of tobacco after potatoes and after corn. From the standpoint of parasitic disease as a factor, it will be recalled that tobacco and potatoes belong to the same family. From the standpoint of nutrition requirements, the corn crop removes much larger quantities of plant nutrients from the soil than does the potato crop. The omission of either nitrogen, phosphorus, or potassium from the fertilizer has materially reduced the yield, the omission of phosphorus having the greatest effect, and of nitrogen the least (pl. 5). The fertilizer effect has been greater than the crop effect, but the former does not overcome the latter. In view of the definite response to even the comparatively small additions of fertilizers made here, it is not clear why the potato crop does not produce a decided increase in yield of tobacco as compared with the effect of the corn crop. It may be added that for 1924, with a very wet spring, the growth of tobacco after corn has been very poor as compared with the growth after potatoes and after tobacco itself, especially where a complete fertilizer is not used. It may be, therefore, that earlier results will soon be reversed.

The yields of potatoes have been quite small, but some interesting differences have been obtained. Recalling the relationships between tobacco, potatoes, and corn as bearing on plant-disease factors and on comparative withdrawal of plant nutrients from the soil, it will be seen that the yield of potatoes is considerably better after tobacco than when potatoes are the preceding crop. On the other hand, continuous culture gives decidedly better yields than are obtained after corn. Moreover, the yield after tobacco is almost double that after corn. In this instance fertilizers apparently have not greatly influenced the result, the crop effects being much greater than the fertilizer effects. In continuous culture the tubers are severely affected with scab while there is little evidence of the disease after tobacco and corn. The average yields of tobacco on the control plots for potatoes in continuous culture and in rotation with tobacco and with corn are, respectively, 921, 883, and 868 pounds

A.—Growth of tobacco after corn on Field I in 1924. On center plot of three rows, potash is omitted from the fertilizer. On plot at left phosphorus is omitted from the fertilizer. Compare growth on these plots with that on the corresponding plots of tobacco after potatoes (B). Plot at right is tobacco after potatoes, the plot receiving a fertilizer supplying nitrogen, phosphorus, and potassium

B.—Growth of tobacco after potatoes on Field I in 1924. On center plot of three rows potash is omitted from the fertilizer. On plot at left phosphorus is omitted from the fertilizer. Growth of the tobacco is much better than after corn under similar conditions (A). Plot at right is tobacco after tobacco, the plot receiving nitrogen, phosphorus, and potassium in the fertilizer. The growth of tobacco, both after potatoes and after tobacco, is much better than after corn. The injurious effect of corn on the growth of tobacco is not always overcome by liberal fertilizing

per acre, indicating an approximately equal productiveness as a whole for the plots involved. The trend of the yields under the three cropping systems for the period covered is shown in Figure 5, which makes clear the progressive increase in yields after tobacco as compared with results after corn. The corn crop, which is in no wise related to potatoes, seems to produce an unfavorable influence on the production of tubers which is not materially modified by any of the fertilizer treatments used.

Good yields of corn have been maintained during the period of the tests, the yields of the last year being but little below those of the first. The average yields of tobacco on the control plots for corn in continuous culture, in culture are due at least in part to original lower productivity of the soil. Thus far the indications are that corn is less affected by preceding crops than tobacco and potatoes, although other crops are greatly affected by corn. In the rotations with tobacco and potatoes, the fertilizer treatments have had little effect on the corn crop, whereas in continuous corn culture the yields have been materially reduced by omission of each of the three elements from the fertilizer. Where the complete fertilizer mixture is used the differences in yields as between continuous culture and rotation are small and hardly significant.

As regards the bearing of these results on the plant-food theory and the theory of soil toxins, it might be

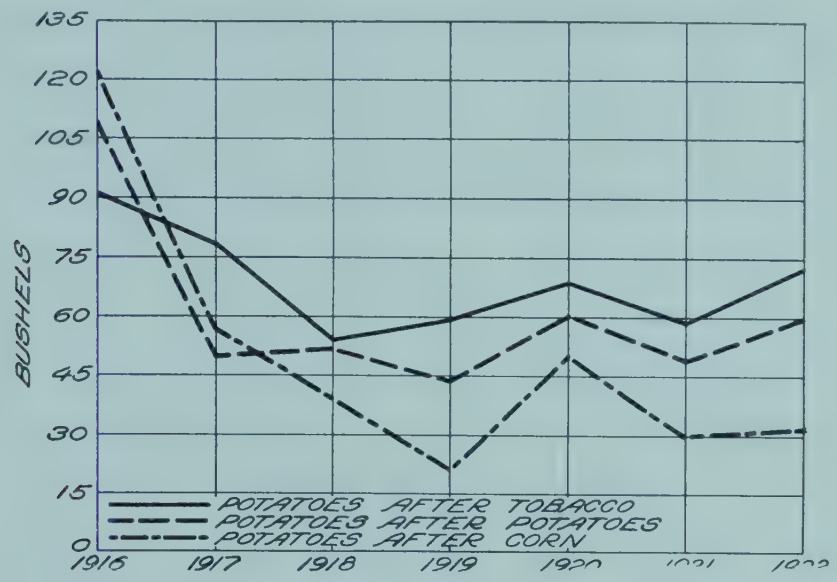

FIG. 5.—Comparative effects of tobacco, potatoes, and corn, as preceding crops, on the yield of potatoes. The "crop effects" evidently are influenced by seasonal conditions, but the yield of potatoes has been somewhat better after tobacco than after potatoes, and much better after tobacco than after corn. It appears that these differences are increasing. The yields here shown are the averages of all fertilizer treatments

rotation with tobacco and in rotation with potatoes, are, respectively, 823, 905, and 868 pounds of leaf, indicating that the productiveness of the plots occupied continuously by corn is somewhat less than the average of the plots occupied alternately by tobacco and corn. The data in Table IV indicate that this difference is due entirely to the superiority of the cropping unit represented by plot 13 of Section A over that represented by plot 13 of section C, the yields of the latter and of plot 3 of Section C being the same. It should be stated, also, that the differences in corn yields on the two cropping units have remained approximately constant from year to year. It seems probable that the reduced yields of corn in continuous

expected, perhaps, that in comparison with the tobacco-cropping tests already discussed and the rotation tests which follow, the present series, which includes only hoed crops, would sooner or later involve more particularly the phenomenon of general soil exhaustion due to heavy annual withdrawals of plant nutrients. It is generally considered that soil toxins of vegetable origin are, for the most part, transitory products, so that intervals of several months each year when the soil is not occupied by the crops, such as occur in this series, would tend to reduce injurious effects on succeeding crops from this cause. Moreover, broadly speaking, the toxic effects of any particular crop tend to become evident in the early years of cropping, and these

effects would be cumulative only to a limited degree. However, depletion of plant nutrients ordinarily would be essentially progressive over relatively long periods and might first become of importance after several years' cropping. So far, the expectations just mentioned seem to have been realized in part, but there are important exceptions. Certain of the crop effects are not influenced at all or are only partly overcome by the fertilizer treatments used and are not in accord with the facts as to comparative withdrawals of plant nutrients by the different crops. It will be of interest to ascertain the result of continued soil depletion by prolonged cropping for comparison with the more immediate crop effects which do not seem to be explainable by the plant-food theory alone. No definite conclusion can be reached at this time as to the true nature of these latter effects.

TOBACCO, POTATOES, AND CORN IN ROTATION WITH WHEAT, OATS, AND RYE

These cropping systems constitute the second step in testing the fundamental value of the general type of rotation under study and should bring out the relative merits of the various possible combinations of these two groups of crops. In this series a deep-rooted crop regularly alternates with a shallow-rooted crop. Mention was made in the preceding series of the fact that tobacco and potatoes belong to the same family, and in the present tests it is well to keep in mind that corn is similarly related to wheat, oats, and rye. There is a comparatively long interval between the harvest of the small grain crops and the planting of the hoed crops which follow, the soil thus remaining unoccupied for nearly 12 months. The small grains, on the other hand, are seeded almost immediately after harvest of the hoed crops.

The general plan of the cropping tests and the fertilizer treatments are quite similar to those used in the preceding tests, except that two fields are employed instead of one in order that all crops may be grown each year. The fields are numbered II and III. Beginning with 1914 on Field II, tobacco, potatoes, and corn have been grown in even years, and wheat, oats, and rye in odd years. On Field III, beginning with 1915, the hoed crops have been grown in odd years and the small grains in even years. The location of the fields and the general characteristics of the soil are shown on the soil map and accompanying report (p. 1199). The detailed plans of Field II, the cropping system, and the fertilizer treatments are shown in Figure 6. The plan and treatments of Field III are the same, except that the dimensions of the plots differ slightly from those of Field II. As in the experiments previously described, tobacco is grown on Section A, potatoes on Section B, and corn on Section C. Following these crops, wheat is seeded on Division 1, embracing the first five plots of Sections A, B, and C. Similarly, oats are seeded on Division 2, extending across the three sections and embracing Plots 6, 7, 9, and 10, Plot 8 being a control; and rye is seeded on Division 3, embracing Plots 11, 12, 14, and 15 of the three sections, Plot 13 being a control.

Maryland Broadleaf tobacco, Irish Cobbler potatoes, and Boone County White corn have been used in the tests. For the winter small grains Currell's Prolific wheat, Culberson oats, and local seed of winter rye have been used. The rates of seeding have been 2 bushels of oats and 1½ bushels of wheat and rye per acre. As indicated in Figure 6, the fertilizers have been applied to the hoed crops at the normal rates throughout the tests. The first crop of wheat, oats, and rye on both fields received no fertilizer, but all subsequent crops of small grains have received one-half the normal rates applied to the hoed crops. Liming has been the same as on Field I. There has been very little winter killing of rye and wheat, but in a few instances oats have been somewhat damaged by this factor, the injury being mostly limited to oats after corn where but little fall growth had been made. During the first three years of the experiments, considerable injury to wheat resulted from joint worm, but subsequently this has been avoided by plowing under the grain stubble shortly after harvest and removing all wheat straw from the farm immediately after threshing.

Each year the stand of potatoes on both fields has been nearly perfect. On Field II the tobacco crop of 1922 was practically a failure because of the wildfire and mosaic diseases and has not been included in tabulating the average yields. In 1916, 1918, and 1920 the stands of tobacco on the three cropping units of Field II ranged from 85 to 96 per cent, and the differences in any year did not exceed 6 per cent. On Field III the stands of tobacco have been 95 per cent or better.

FIG. 6.—Plan of Field II, showing the arrangement and dimensions of plots and intervening spaces, the fertilizer treatments, and the cropping system

TABLE VI.—*Comparative productiveness of the cropping units of Field II as indicated by the average yields of tobacco and wheat on the control plots for the period 1915 to 1923, inclusive*

Location of plot	Tobacco						Wheat					
	Yields of leaf, per acre			Yields of stalks, per acre			Yields of grain, per acre			Yields of straw, per acre		
	Plot 13	Plot 8	Plot 3	Plot 13	Plot 8	Plot 3	Plot 13	Plot 8	Plot 3	Plot 13	Plot 8	Plot 3
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Bush.*	*Bush.*	*Bush.*	*Lbs.*	*Lbs.*	*Lbs.*
Section A	756	853	880	408	488	497	7.5	9.4	12.0	870	1,058	1,245
Section B	745	763	895	421	429	449	9.3	10.3	13.8	975	1,255	1,410
Section C	719	640	952	407	368	545	7.0	8.6	12.0	773	975	1,240

TABLE VII.—*Comparative productiveness of the cropping units of Field III as indicated by the average yields of tobacco and wheat on the control plots for the period 1916 to 1922*

Location of plot	Tobacco						Wheat					
	Yields of leaf, per acre			Yields of stalks, per acre			Yields of grain, per acre			Yields of straw, per acre		
	Plot 13	Plot 8	Plot 3	Plot 13	Plot 8	Plot 3	Plot 13	Plot 8	Plot 3	Plot 13	Plot 8	Plot 3
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Bush.*	*Bush.*	*Bush.*	*Lbs.*	*Lbs.*	*Lbs.*
Section A	1,076	1,122	1,082	626	698	604	9.4	9.8	10.8	1,225	1,344	1,545
Section B	931	906	973	469	466	458	7.7	8.7	9.8	1,299	1,216	1,592
Section C	844	859	863	445	465	397	10.0	9.7	10.5	1,317	1,363	1,476

The stand of corn has been excellent on both fields. On Field III the immature ears of corn were badly damaged by birds in 1915 and the yields of grain were computed on the data from uninjured plants constituting 50 to 60 per cent of the total. Stands of small grains have been uniformly good.

The arrangement of cropping control plots is the same as for Field I. Those of Field II received the complete fertilizer at the normal rate in 1916 and 1918, and those of Field III were similarly treated in 1915 and 1917. In other years no fertilizers have been applied to these plots. The control plots throughout the two fields have been cropped in tobacco and wheat in regular rotation. The average yields are summarized in Tables VI and VII. Because of serious injury from joint worm the yields of wheat on Field III in 1918 are not included in the averages.

One of the facts brought out in Tables VI and VII which should be mentioned is that tobacco and wheat as indicators of relative productivity of different soil areas do not always give similar results. Thus, the yields of wheat on Field II indicate a greater superiority in productiveness of Division 1, as represented by Plot 3, over Divisions 2 and 3, represented by Plots 8 and 13, than is indicated by the yields of tobacco on the same plots. Again, on Field III the yields of tobacco would indicate that Section A as a whole is considerably more productive than Section C. This is contrary to the results with wheat, and the general lay of the land and the character of the soil indicate that for most staple crops Section C is at least equal, if not superior to Section A in productivity. Under the circumstances it seems logical to depend primarily on results with wheat in dealing with small grains, but to rely more on results with tobacco in considering the hoed crops.

The average results obtained on Fields II and III with the small grains after each of the three hoed crops are summarized in Table VIII. In this table it is a simple matter to compare the effects of the different hoed crops on each of the small-grain crops and also compare these crop effects with the effects of the different fertilizer treatments.

Taking first the wheat crop, the average yields of wheat on the control plots for the wheat division (Plot 3) of Sections A, B, and C of the two fields are 11.4, 11.8, and 11.3 bushels, respectively, indicating substantial uniformity in productivity of the cropping units involved. It appears, therefore, that corn has had a decidedly depressing action on yield of both grain and straw of wheat, while there is only a slight difference in the effects of tobacco and potatoes. These relations hold true under all the fertilizer treatments used. The fertilizer treatments also clearly affect the yields of wheat, omission of phosphorus having a markedly depressing action. It is interesting to note that although the fertilizer treatments influence the wheat yields in any particular case, they show no well-defined tendency to overcome the differences in effects of the preceding crops of tobacco, potatoes, and corn.

Turning to the oats crop, the average yields of wheat on the control plots for the oats division (Plot 8) of the sections of the two fields occupied respectively by tobacco, potatoes, and corn are 9.6, 9.5, and 9.2 bushels. These yields stand at a somewhat lower level than those for the wheat division of the fields, already discussed, but these yields agree satisfactorily among themselves, indicating approximate uniformity in productivity of the cropping units occupied by oats. Here, again, the depressing effect of corn on the yields of oats is noticeable, and there is also an appreciable gain in yield after potatoes as compared with the yield after tobacco. The fertilizer treatments have affected the yields, but these effects are not sharply defined in all cases. In this instance omission of nitrogen from the fertilizer rather than phosphorus has had the greatest depressing effect, although after corn omission of phosphorus seems to be of significance. As in the case of wheat, the fertilizer treatments do not accomplish much in overcoming the differences in effects of the three preceding hoed crops.

Considering finally the rye crop, the average yield of wheat on the control plots of the rye division (Plot 13) of the two fields is 8.5 bushels alike for the tobacco, potatoes, and corn sections. Potatoes as a preceding crop seem to favor the growth of rye, the yields of both grain and straw being markedly increased as compared with results after tobacco and corn. The yields after tobacco are only slightly better than those after corn, but the differences seem to be appreciably influenced by the fertilizer treatment. When potassium or phosphorus are omitted from the fertilizer, the differences in yield after tobacco and after corn are increased. With tobacco as the preceding crop, nitrogen seems to be the chief plant nutrient limiting yields,

TABLE VIII.—*Summary of results with small grains in Fields II and III for the years 1915 to 1923, inclusive, showing the comparative effects of tobacco, potatoes, and corn as preceding crops on the yields of wheat, oats, and rye under different fertilizer treatments; also, the effects of the fertilizer treatments on the yields of wheat, oats, and rye*

Fertilizer treatment	Yield of wheat				Yield of oats				Yield of rye			
	After tobacco	After potatoes	After corn	Average	After tobacco	After potatoes	After corn	Average	After tobacco	After potatoes	After corn	Average
	Bush.	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*
½ (N+P)	14.4	14.1	9.5	12.7	21	24.2	16.1	20.4	15.8	19.8	13.2	16.3
½ (N+K)	10.1	9.2	6.6	8.6	19.4	22.7	12.8	18.3	14.3	17.3	12.1	14.6
½ (P+K)	12.0	13.9	9.0	11.6	15.7	18.8	12.9	15.8	11.3	16.7	12.6	13.5
½ (N+P+K)	12.7	13.9	9.1	11.9	16.7	20.9	14.4	17.3	14.1	20.5	14.6	16.4
Average	12.3	12.8	8.6	11.2	18.2	21.6	14.1	18.0	13.9	18.6	13.1	15.2

Fertilizer treatment	Yield of wheat straw				Yield of oats straw				Yield of rye straw			
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
½ (N+P)	1,648	1,765	1,038	1,484	855	1,069	751	892	2,061	2,597	1,551	2,070
½ (N+K)	1,208	1,207	753	1,056	858	971	580	803	1,731	2,191	1,398	1,773
½ (P+K)	1,235	1,611	930	1,259	726	814	551	697	1,552	2,357	1,371	1,760
½ (N+P+K)	1,427	1,639	1,018	1,361	778	912	631	774	1,725	2,651	1,819	2,065
Average	1,379	1,555	935	1,290	804	942	628	791	1,768	2,449	1,535	1,917

while with potatoes and corn as preceding crops nitrogen and phosphorus seem to be of about equal importance. In general, the differences in yield under the different fertilizer treatments are not as great as might be expected.

Summarizing, it appears that corn, in comparison with tobacco and potatoes, has a depressing effect on the yields of wheat, oats, and rye. Compared with tobacco, corn shows the greatest depressing action on wheat and the least on rye. Compared with potatoes, corn reduces the yields of wheat, oats, and rye about the same extent. After tobacco the yield of wheat is about the same as after potatoes, the yield of oats is reduced less than 20 per cent, while the yield of rye is reduced by about a third. These crop effects are not greatly modified by the different fertilizer treatments used. Why the effects of tobacco more nearly resemble those of potatoes rather than those of corn is not clear from the standpoint of the plant-food theory.

The comparative effects of tobacco, potatoes, and corn on each of the small grains for each year as well as the general trend of these crop effects are shown graphically in Figures 7, 8, and 9. The yields shown are the combined weights of grain and straw, and are the averages of all fertilizer treatments. For the most part there is a notable similarity in yields of wheat after tobacco and after potatoes, but in certain years production is much greater after potatoes than after tobacco. On the other hand, with a single exception, the relative growth of wheat after corn is decidedly reduced each year, and in some years the differences are very large. In the earlier years of the test, oats usually gave considerably better results after potatoes than after the other two crops, and the yields after tobacco and corn were about the same, except in 1915. In recent years, however, yields after tobacco have more nearly equaled those after potatoes. Throughout the test, the growth of rye after tobacco has been closely similar though somewhat superior to that after corn. Potatoes as a preceding crop greatly stimulated the growth of rye at first, but in later years this advantage has not been maintained. It is of interest to note that there has been no progressive decline in yields of any of the small grains, even after corn.

The average results with tobacco, potatoes, and corn after the three small grains on Fields II and III are summarized in Table IX. The average yields of tobacco on corresponding control plots of the two fields indicate comparative uniformity in productiveness of the three cropping units of the tobacco sections, while in the potato and corn sections the cropping units occupied by wheat seem to be some-

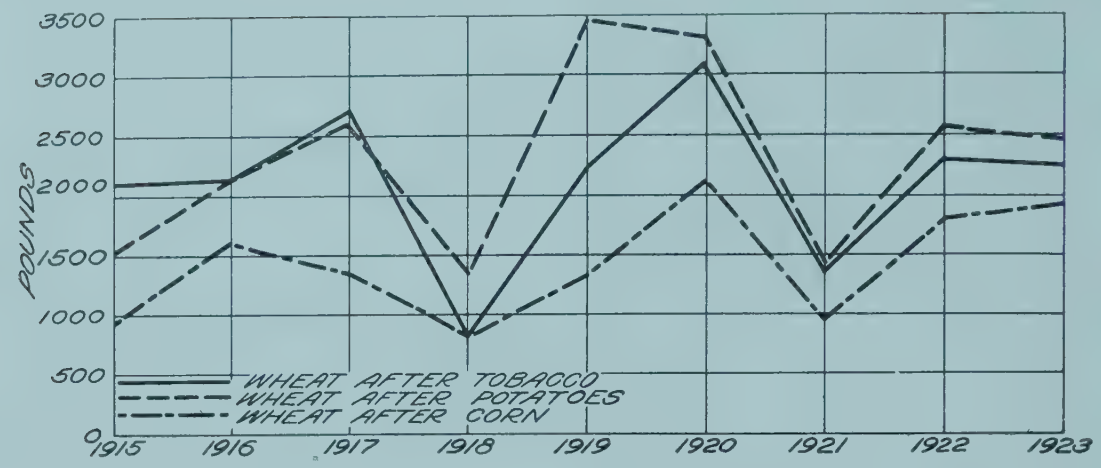

FIG. 7.—Comparative effects of tobacco, potatoes, and corn as preceding crops on the yield of wheat. The comparative results are affected by the seasonal conditions, but in most years the yields after tobacco have not differed greatly from those after potatoes. After corn the yields are lower—frequently much lower—than after tobacco and potatoes. The yields recorded are the combined weights of grain and straw.

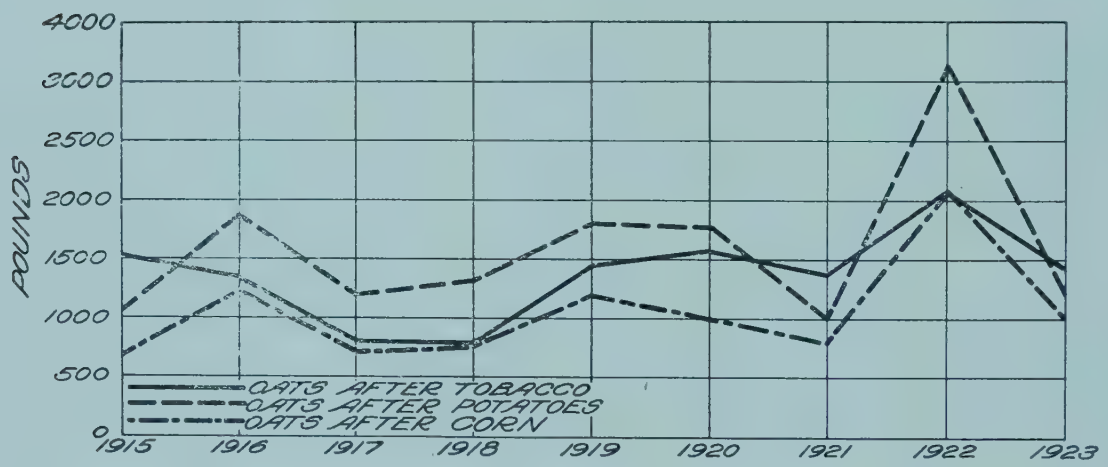

FIG. 8.—Comparative effects of tobacco, potatoes, and corn as preceding crops on the yield of oats. Here, again, the lowest yields are after corn. The yields generally lie between those after potatoes and after corn. The yields recorded are the combined weights of grain and straw

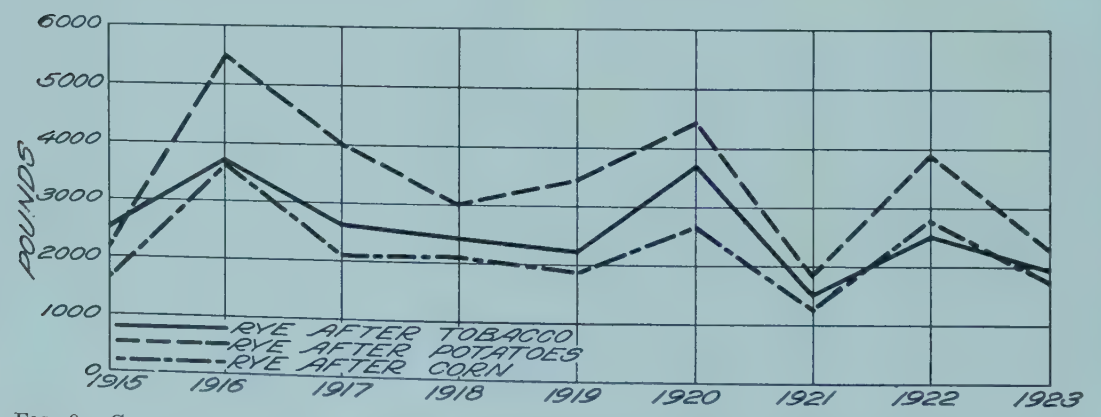

FIG. 9.—Comparative effects of tobacco, potatoes, and corn as preceding crops on the yield of rye. The rye at first seems to thrive particularly well after potatoes, but subsequently the yield more nearly approaches the yields following tobacco and corn. There is less difference in yields of rye after the lattter two crops than in the case of wheat and oats, though the results after tobacco are somewhat better than those after corn. The yields are expressed as the combined weights of grain and straw

what more productive than those occupied by oats and rye. These latter differences are due to apparent soil variation in Field II, there being no substantial differences in yields of the three control plots in any of the sections of Field III. As a whole, the differences in effects of the three small grain crops on the hoed crops are comparatively small, possibly because there is a rather long interval between the time of harvest of the former and planting of the latter, coupled with the fact that the small grains are shallow-rooted crops. As to tobacco, there seems to be no difference in the effects of wheat and oats as preceding crops. Taking the average of all fertilizer treatments as shown in Figure 10, the tobacco yields after the two crops have agreed closely each year on the two fields. Rye, as compared with wheat and otas, appears to have a depressing effect which is not overcome or greatly modified by any of the fertilizer treatments, except that on Field III the complete fertilizer does overcome this effect. There is some indication in Figure 10 that this effect is progressive. Of the three fertilizing elements, nitrogen seemed to have the greatest effect on the yield of tobacco, while potassium has seemed to have had little effect.

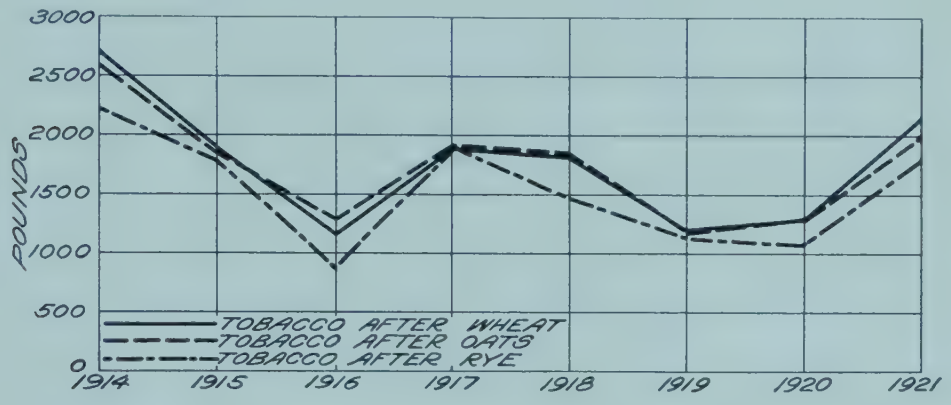

FIG. 10.—Comparative effects of wheat, oats, and rye as preceding crops on the yield of tobacco. In general the differences in effects of the small grains on the hoed crops are not great. The yields of tobacco after wheat and after oats closely agree each year. Rye, in comparison, shows a slight depressing effect. The yields are expressed as the combined weights of leaf and stalk

TABLE IX.—*Summary of results with hoed crops on Fields II and III for the years 1916 to 1922, inclusive, showing the comparative effects of wheat, oats, and rye as preceding crops on the yields of tobacco, potatoes, and corn, under different fertilizer treatments; also, the effects of the fertilizer treatments on the yields of tobacco, potatoes, and corn.*

Fertilizer treatment	Yield of tobacco leaf				Yield of potatoes				Yield of corn			
	After wheat	After oats	After rye	Average	After wheat	After oats	After rye	Average	After wheat	After oats	After rye	Average
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*
N+P	1,122	1,053	912	1,029	64.1	54.1	50.8	56.3	43.9	38.9	33.4	38.7
N+K	1,042	959	939	980	48	42.5	39.1	43.2	42.1	35.8	35.7	37.9
P+K	861	914	750	842	53.1	48.3	39.6	47	35.9	31.4	26.6	31.3
N+P+K	1,091	1,087	959	1,046	72	69.4	57.8	66.4	38.4	35.7	34.7	36.3
Average	1,029	1,003	891	974	59.3	53.6	46.8	53.2	40.1	35.5	32.6	36.1

Fertilizer treatment	Yield of tobacco stalks				Yield of corn stover			
	After wheat	After oats	After rye	Average	After wheat	After oats	After rye	Average
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
N+P	645	629	516	597	3,172	2,783	2,533	2,829
N+K	544	551	521	539	2,903	2,404	2,429	2,579
P+K	457	498	357	437	2,515	2,298	2,250	2,354
N+P+K	648	686	562	632	2,975	2,755	2,769	2,833
Average	573	591	488	551	2,891	2,560	2,496	2,649

The yields of potatoes are small and it is doubtful if the apparent crop effects are significant. Phosphorus and nitrogen are the most significant fertilizing elements. The yields of corn on Field II have rapidly declined in later years after oats and especially after rye, but this result is probably due in part to original differences in productiveness of the soil. On Field III no differences have developed thus far in the effects of wheat, oats, and rye on the yields of corn. Nitrogen is the important fertilizing element in the corn yields.

ments being applied. The two fields used are IV and V. The location and the general characteristics of the soil of the two fields are shown on the soil map and accompanying report. The plan of Field IV, together with the detailed system of cropping, are shown in Figure 11. The plan of Field V is the same in all respects, except that lack of a sufficiently large area of suitable soil made it necessary to locate the rye division apart from the remainder of the field and immediately adjacent to Field IV, as shown on the soil map.

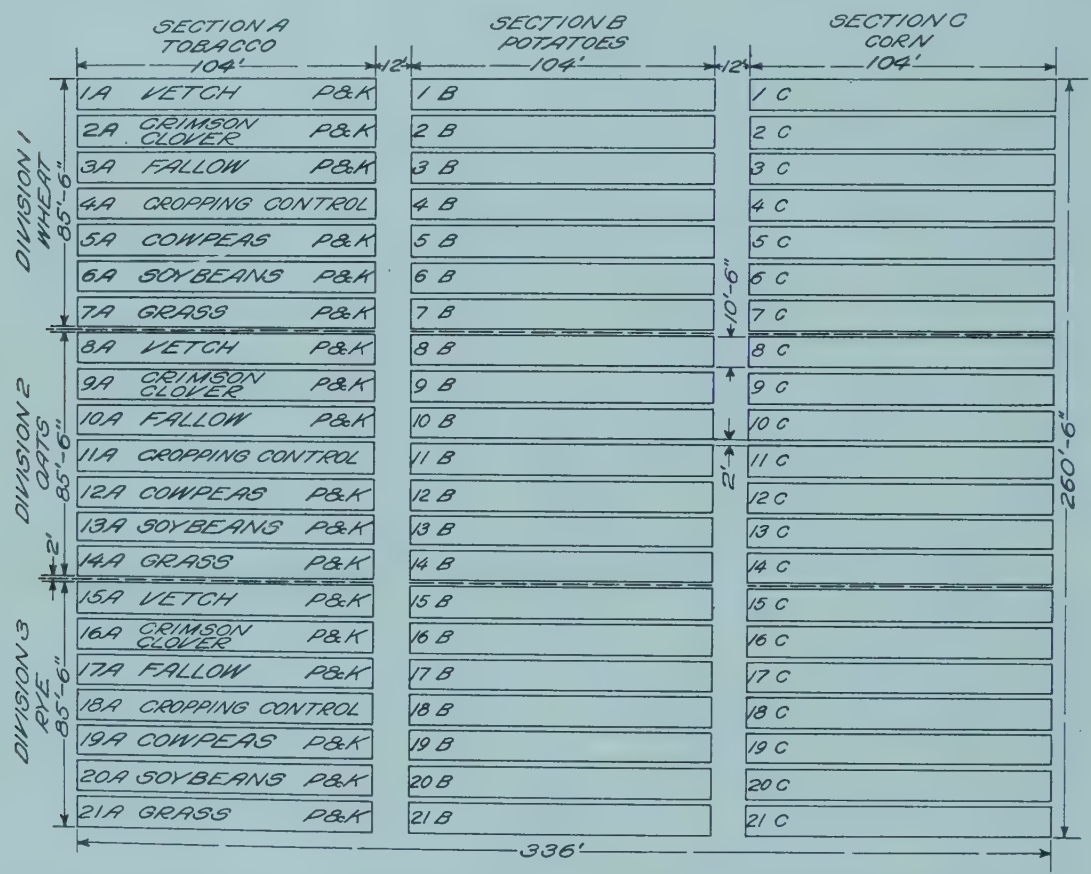

FIG. 11.—Plan of Field IV showing the arrangement and dimensions of the plots and intervening spaces and the cropping system followed. The grass and legumes are used as soiling crops

TOBACCO, POTATOES, AND CORN IN ROTATION WITH SMALL GRAINS AND LEGUMES

Addition of legumes to the cropping systems last discussed constitutes the final step in testing the value of the type of rotation under study and should throw more light on the relative merits of the several possible combinations of hoed crops and small grains included in the tests. These tests are essentially repetitions of the previously described tests with hoed crops in rotation with small grains, with the important difference that grass and various legumes are grown as soiling crops on the plots of each cropping unit instead of different fertilizer treat-

As in the preceding tests, tobacco, potatoes, and corn are grown in parallel strips, followed by wheat, oats, and rye seeded crosswise these strips. Each of the nine cropping units thus formed is subdivided into seven plots, of which one is used as a cropping control. Of the remaining plots, one receives no additional crop, while the others are seeded to hairy vetch, crimson clover, cowpeas, soy beans, and a grass mixture, respectively. In this way the effects of two summer legumes and two winter legumes as annual soiling crops are compared with a non-leguminous soiling crop and with fallowing. The summer legumes are planted shortly after harvesting the small grains and the other soiling crops

are seeded in early fall. All plots are fertilized uniformly, with the precipitated dicalcic phosphate and sulphate of potash at the standard rates of 40 pounds phosphoric acid and 20 pounds potash per acre, respectively, for the hoed crops, and beginning with 1918, one-half these rates have been applied each year to the small grains. No nitrogen is applied to any of the plots. Liming has been the same as on Fields II and III.

Tobacco, potatoes, and corn follow directly after the soiling crops, while the small grains are subject only to the residual effects of the soiling crops. As a nonleguminous cover crop a mixture of timothy, tall meadow oat, and orchard and Italian rye grass is used. Beginning with 1916 the hoed crops have been grown on Field IV in even years and on Field V in odd years. Legumes were first seeded on Field IV in 1917 after the small grains, and on Field V they were planted in 1916 in preparation for the first crops of tobacco, potatoes, and corn. Only the cowpeas showed effective inoculation in the crop of the first year, but all legumes have made good growth since the first year. Stands of the various crops have been satisfactory in nearly all cases.

The arrangement and treatment of cropping control plots are the same as for Fields II and III, and tobacco and wheat have been regularly grown in rotation on the plots. The average yields are shown in Tables X and XI. The data for wheat in 1918 on Field V are unreliable, because of damage from joint worm, and are not included in the averages. As was found to be true on Fields II and III, the data in these tables disclose several instances in which tobacco and wheat as indicators of relative soil productiveness give contradictory results. The most striking case is that of the rye division of Field V, represented by Plot 18 of the three sections, as compared with Plots 11 and 4, representing the oats and wheat divisions, respectively. The results with tobacco indicate that the rye division is the more productive, but the yields of wheat on the control plots are much lower than on the control plots of the oats and wheat divisions. On the whole, Field IV seems to be comparatively uniform except that the tobacco-rye cropping unit represented by Plot 18 is undoubtedly somewhat less productive than the remainder of the field. On Field V the contradictory results as to the rye division have been mentioned already. The results with tobacco and wheat agree in indicating that the corn section (C) is some 20 to 25 per cent less productive than the remainder of Field V.

TABLE X.—*Comparative productiveness of the cropping units of Field IV as indicated by the average yields of tobacco and wheat on the control plots for the period 1916–1922, inclusive*

Location of plot	Tobacco						Wheat					
	Yields of leaf per acre			Yields of stalks per acre			Yields of grain per acre			Yields of straw per acre		
	Plot 18	Plot 11	Plot 4	Plot 18	Plot 11	Plot 4	Plot 18	Plot 11	Plot 4	Plot 18	Plot 11	Plot 4
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Bush.*	*Bush.*	*Bush.*	*Lbs.*	*Lbs.*	*Lbs.*
Section A	891	1,025	1,035	520	624	659	10.3	13.2	14	1,303	1,873	1,507
Section B	1,110	1,143	1,100	655	700	775	14	12	13.7	2,123	1,927	1,767
Section C	950	813	899	610	606	698	12.7	13.3	16.3	1,807	1,790	1,933

TABLE XI.—*Comparative productiveness of the cropping units of Field V as indicated by the average yields of tobacco and wheat on the control plots for the period 1917–1922, inclusive*

Location of plot	Tobacco						Wheat					
	Yield of leaf per acre			Yield of stalks per acre			Yield of grain per acre			Yield of straw per acre		
	Plot 18	Plot 11	Plot 4	Plot 18	Plot 11	Plot 4	Plot 18	Plot 11	Plot 4	Plot 18	Plot 11	Plot 4
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Bush.*	*Bush.*	*Bush.*	*Lbs.*	*Lbs.*	*Lbs.*
Section A	1,041	869	916	450	422	458	9.8	15.8	17.8	980	1,600	1,810
Section B	1,017	721	842	497	381	394	10	17.5	17.5	1,050	2,190	1,930
Section C	754	713	696	460	365	407	8.5	11.8	14.8	850	1,150	1,360

Summaries of the results with tobacco, potatoes, and corn on Fields IV and V are given in Table XII. Two features are of interest, namely: (1) The comparative effects of preceding crops of wheat, oats, and rye as modified by the intervening soiling crops; and (2) the comparative, direct effects of the soiling crops on tobacco, potatoes, and corn. As to the first-named feature, the results with tobacco are quite similar to those obtained in the preceding tests where no soiling crops were used. Although the differences in effects of wheat, oats, and rye are not large, these differences seem to be significant, particularly as to the depressing effect of rye on the yields of tobacco. Thus, in 1917, the first year of the test, the average yield of tobacco on the rye division of Field V was 1,340 pounds per acre and on the wheat division the yield was 1,320 pounds. In 1921 the corresponding yields were 1,008 pounds and 1,245 pounds per acre. The general effect of the intervening legumes has been to intensify the relatively unfavorable effect of rye on the growth of tobacco. Grass as a soiling crop, on the other hand, seems to act in the reverse manner, giving somewhat better results after rye than after wheat. These effects of the legumes are associated with a tendency toward increased yields of tobacco, while the effects of grass are associated with decreased yields.

The direct effects of the soiling crops on tobacco are interesting in several particulars. For the comparatively short period of the tests, vetch, crimson clover, and cowpeas as soiling crops have given rather large increases of tobacco where wheat was the preceding crop, just as was the case in the early years of the special cropping tests with tobacco which have already been discussed. With rye as the preceding crop, and to a lesser extent with oats, these legumes have been much less effective in increasing the yield of tobacco. Of the four legumes, soy beans are unique in that they are wholly ineffective in all cases in promoting the growth of tobacco and in some instances have actually decreased the tobacco yield. Grass as a soiling crop has had a remarkable depressing action on the growth of tobacco, the yields of which are reduced to nearly half those of the fallow plots. These relationships are brought out in more detail in Figure 12.

TABLE XII.—*Summary of results with hoed crops on Fields IV and V for the years 1918–1922, inclusive, showing the comparative effects of wheat, oats, and rye as preceding crops on the yields of tobacco, potatoes, and corn, when soiling crops intervene in the rotation; also the comparative effects of the soiling crops on the yields of tobacco, potatoes, and corn*

Soiling crop grown	Yield of tobacco leaf				Yield of potatoes				Yield of corn			
	After wheat	After oats	After rye	Average	After wheat	After oats	After rye	Average	After wheat	After oats	After rye	Average
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*
Vetch	1,352	1,041	858	1,084	74.8	56.1	53.1	61.3	59.1	59.9	53.4	57.5
Crimson clover	1,410	1,181	969	1,187	77.8	52.5	51.5	60.6	59.9	54.9	49.5	54.8
Fallow	920	999	799	906	59.8	51.6	46.4	52.6	41.4	42.9	42.0	42.1
Cowpeas	1,321	1,299	1,156	1,259	71.1	60	65.9	65.7	58.0	53.5	55.8	55.8
Soy beans	938	822	846	869	54.1	45.8	52.1	50.7	54.5	49.5	54.4	52.8
Grass	489	668	563	573	38.7	31.2	39.5	36.5	37.8	35.1	42.5	38.5
Average	1,071	1,002	865	979	62.7	49.5	51.4	54.6	51.8	49.3	49.6	50.2

Soiling crop grown	Yield of tobacco stalks				Yield of corn stover			
	After wheat	After oats	After rye	Average	After wheat	After oats	After rye	Average
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
Vetch	784	499	404	562	4,290	4,120	3,635	4,015
Crimson clover	776	595	493	621	4,287	3,899	3,470	3,885
Fallow	514	517	369	467	3,230	3,207	2,962	3,133
Cowpeas	729	657	624	670	3,930	3,744	3,914	3,863
Soy beans	468	392	403	421	3,643	3,349	3,685	3,559
Grass	219	304	249	257	2,605	2,597	3,001	2,734
Average	582	494	424	500	3,664	3,486	3,443	3,531

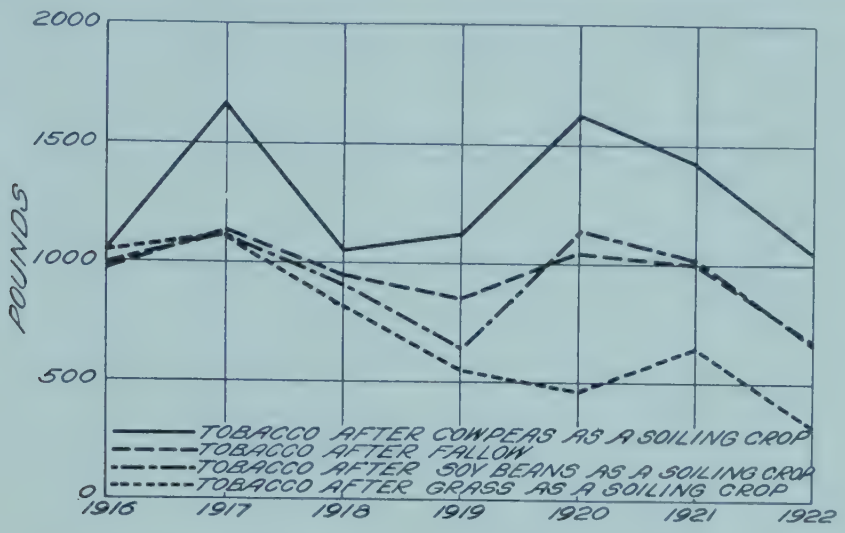

FIG. 12.—Effects of cowpeas, soy beans, and grass as soiling crops on the average yield of tobacco in the rotations with wheat, oats, and rye. For the comparatively short period covered, cowpeas show a favorable, though variable, effect on the growth of tobacco. Soy beans, on the other hand, have failed from the outset to produce decided increase in growth and in some years they have actually depressed the yield. Grass as a cover crop has caused a marked downward trend in the tobacco yield

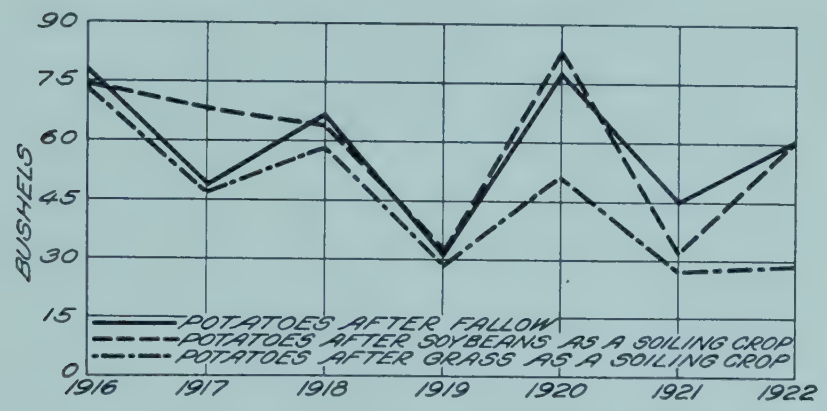

FIG. 13.—Effects of soy beans and grass as soiling crops on the average yield of potatoes in the rotations with wheat, oats, and rye. The results are similar to those with tobacco, as shown in Figure 12. Soy beans in the beginning showed a beneficial effect on potatoes, but in later years the effect has been either nil or injurious. Grass as a cover crop has resulted in a decided downward trend in yield of potatoes

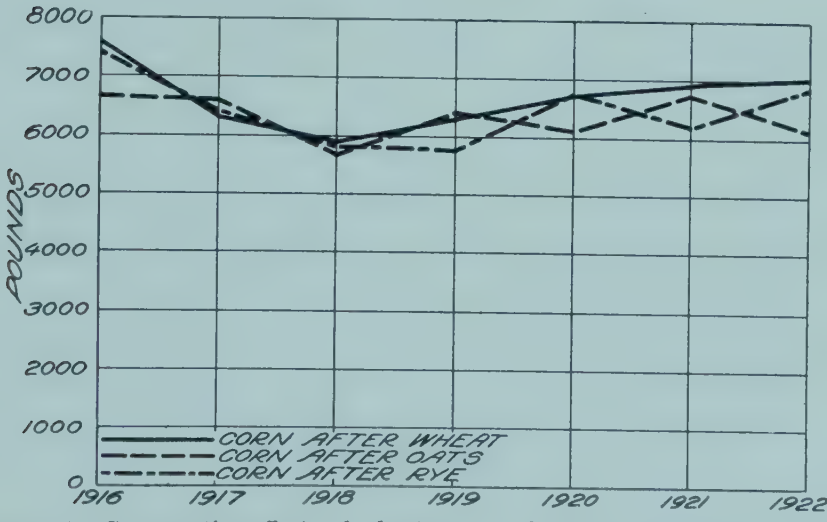

FIG. 14.—Comparative effects of wheat, oats, and rye on the corn crop when intervening soiling crops are included in the rotations. There is a notable similarity in the effects of the three small grains, although, on the whole, wheat appears to exert a somewhat more favorable action than oats or rye. The upward trend in yield since 1918 is considered due to the action of the soiling crops. This result differs from the results with tobacco and potatoes shown in part in Figures 12 and 13. The yields of corn are the combined weights of grain and straw

In the case of potatoes, the results generally are similar to those with tobacco except that, as compared with wheat, oats as well as rye have a depressing effect on yields. Vetch, crimson clover, and cowpeas are of little value in increasing the yields of potatoes when either oats or rye is the preceding crop. Soy beans have failed to increase yields even with wheat as the preceding crop. Grass has greatly depressed the potato yields in all cases. The effects of soy beans and grass on the yields of potatoes are shown graphically in Figure 13. There are no significant general differences in yields of corn with wheat, oats, and rye as preceding crops, and legumes generally are effective in increasing the yield. The similarity in effects of the three small grains are shown in graphic form in Figure 14. Soy beans give almost as good results as the other legumes. Grass depresses yield, but to a lesser extent than is the case with tobacco and potatoes.

Some of these results are difficult to interpret. As in the preceding tests, in which no soil-improving crops were used, tobacco behaves more like potatoes than corn in its response to the influence of preceding crops. Why intervening leguminous crops intensify rather than dissipate the differences in effects of wheat, oats, and rye as preceding crops is not clear. Judged by the older tests previously discussed, the initial increases in yields of tobacco resulting from the addition of legumes to the rotation may be expected to be followed by declining yields. The depressing effect of vetch on the growth of tobacco, in fact, is plainly indicated in the crop of 1924. One of the surprising results is the inability of tobacco and potatoes to effectively utilize the nitrogen supplied by soy beans. Corn apparently appropriates the nitrogen from the soy beans almost, if not quite, as readily as that from other legumes.

It seems possible that the unfavorable effect of grass on the hoed crops is due in part to some factor other than those concerned in the preceding crop effects, for this effect extends to all of the three hoed crops. Each of these crops following grass has shown a yellowish-green color suggestive of nitrogen hunger, and it is possible that this is one of the important factors involved. Bizzell (*2*) has investigated this phase of the effect of crops on those following in the rotation. It has been observed, also, that liming partially overcomes the unfavorable effect of grass. Interference with the nitrogen supply, however, is not the only way in which grass may depress the growth of tobacco. It has been the experience of growers in the Connecticut Valley that tobacco frequently makes very poor growth for the first year or two following timothy sod, even though heavy applications of nitrogenous fertilizers are made. The part played by the grass here may be similar to that of the legumes.

It remains to consider the effects of introducing the soiling crops into the rotation on the small grains following after the hoed crops. The data are summarized in Table XIII.

TABLE XIII.—*Summary of results with small grains on Fields IV and V for the years 1917–1923, inclusive, showing the comparative effects of tobacco, potatoes, and corn as preceding crops on the yields of wheat, oats, and rye, when soiling crops are included in the rotation; also the residual effects of the soiling crops on the yields of wheat, oats, and rye*

Soiling crop grown	Yield of wheat				Yield of oats				Yield of rye			
	After tobacco	After potatoes	After corn	Average	After tobacco	After potatoes	After corn	Average	After tobacco	After potatoes	After corn	Average
	Bush.	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*	*Bush.*
Vetch	17.2	18.1	13.2	16.2	44.2	54.5	25.5	41.4	23.9	28.3	14.7	22.3
Crimson clover	16.9	18.6	13.2	16.2	42.6	53.4	23.3	39.8	18.5	25.4	12.1	18.7
Fallow	13.1	17.5	10.3	13.6	27.2	37.9	15.1	26.7	15.8	21.7	12.3	16.6
Cowpeas	15.5	21.0	13.0	16.5	42.6	48.2	18.6	36.5	18.2	26.1	15.6	20.0
Soy beans	17.0	22.1	10.5	16.5	37.8	42.9	19.4	33.4	18.3	22.4	14.1	18.3
Grass	13.3	18.3	9.1	13.6	27.6	32.2	14.8	25.2	17.3	21.9	13.3	17.5
Average	15.5	19.3	11.6	15.4	37.0	45.0	19.5	33.8	18.7	24.3	13.7	18.9

Soiling crop grown	Yield of wheat straw				Yield of oats straw				Yield of rye straw			
	Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
Vetch	1,972	2,358	1,572	1,967	2,332	3,237	1,196	2,255	2,980	3,770	1,866	2,872
Crimson clover	2,019	2,485	1,404	1,970	1,886	2,694	829	1,803	2,187	3,382	1,311	2,293
Fallow	1,306	1,938	966	1,403	1,126	1,631	632	1,130	1,806	2,934	1,335	2,025
Cowpeas	1,809	2,714	1,329	1,951	1,800	2,083	780	1,554	2,437	3,627	1,876	2,647
Soy beans	1,873	2,777	1,133	1,927	1,766	1,806	690	1,421	2,277	3,138	1,653	2,356
Grass	1,399	2,285	837	1,508	1,103	1,489	647	1,080	2,047	2,938	1,543	2,176
Average	1,730	2,426	1,207	1,788	1,660	2,157	796	1,541	2,289	3,298	1,597	2,395

Comparing the results collectively with those in the rotations of hoed crops and small grains without soiling crops (Tables XIII and VIII), it is apparent that, qualitatively, the relative effects of tobacco, potatoes, and corn remain the same. On the other hand, there are some very striking quantitative differences in the two series. The general effect of the legumes has been to intensify the differences in effects of the three hoed crops on the yields of succeeding crops of small grains. It is an interesting fact that in both series of tests the comparative average effects of tobacco, potatoes, and corn are practically the same for the straw as for the grain of the wheat, oats, and rye. In other words, these crop effects apply to the growth of the plant as a whole rather than that of particular parts and in this way differ somewhat from the effects produced by the fertilizer treatments (Table VIII). The large increases in yields of small grains resulting from the residual effects of the legumes emphasize the fact that the latter have furnished an ample potential supply of nitrogen for the hoed crops, even in the case of soy beans.

The yields of wheat on the control plots indicate that on Field IV the soil of the corn section is somewhat more productive than that of the tobacco and potato sections, while on Field V the reverse appears to be true. Making due allowance for these soil irregularities, it is apparent that corn, in comparison with tobacco and potatoes, depresses the yields of all the small grains (pl. 6). The effect of tobacco on oats resembles that of potatoes rather than that of corn, as in the previous tests, while the yields of wheat and rye after tobacco are about midway between the yields after potatoes and after corn. An outstanding feature is the large increase in the yields of oats after tobacco and potatoes resulting from the residual effects of the legumes. In some years these yields have exceeded 80 bushels per acre. Wheat and rye are less responsive to the residual effects of the legumes. The simplest assumption concerning the cause of the marked residual action of the legumes on the small grains after tobacco and potatoes is that the latter crops were unable to make use of the nitrogen supplied by the legumes, thus leaving in the soil considerable supplies for the small grains. Corn, on the other hand, probably has appropriated most of the nitrogen, leaving but little for the wheat, oats, and rye. There is an interesting question, however, as to why oats after tobacco and potatoes are benefited to a far greater extent than are wheat and rye through the residual action of the legumes. It seems that some factor other than the nitrogen supply has limited the growth of the wheat and rye. The comparative effects of tobacco, potatoes, and corn on the yields of the small grains as influenced by the soiling crops are shown graphically in Figures 15, 16, and 17.

As to the individual soiling crops, it appears that all the legumes have been about equally effective as a whole, although in some cases oats and rye have shown larger gains in yield from the winter legumes than from the summer legumes. It is of considerable interest to note that the depressing action of grass on growth of crops immediately following, so plainly shown by tobacco and potatoes and to a lesser extent by corn, does not extend to the succeeding crops of small grains, the yields on the grass plots checking satisfactorily with those on the fallow plots. This indicates that the unfavorable effects of the grass are only temporary.

RELATIVE EFFECTS OF TOPS AND ROOTS OF CERTAIN PLANTS ON GROWTH OF TOBACCO

The results of the preceding field tests show that, as compared with such crop plants as corn, tobacco is quite sensitive to the effects of preceding crops. These crop effects are not due entirely to the action of the preceding crops on the supply of available plant nutrients in the soil. In some cases unfavorable effects are noted from preceding crops, even when their aboveground portions are removed from the field in harvesting. It seemed of interest, therefore, to compare the effects of the tops and roots of some plants on the growth of tobacco, and some preliminary pot and field tests have been made along these lines.

Glazed 6 and 2 gallon earthen jars were used for the pot tests. Soil from the experimental field which had not been previously cropped was used, and no fertilizer was added. With the larger jars 8,330 grams of soil, and for the smaller jars 2,500 grams were used. The moisture content was maintained at approximately optimum. In the larger pots 200 grams of the plant material were incorporated in the soil, and 66 grams in the smaller pots. The tobacco plants were set out on December 26, and harvested March 6. Detailed observations were made on green weights of tops and roots of the

A.—Wheat in rotation with potatoes and crimson clover on Field V, 1922. The growth is far better than in the rotation with corn and crimson clover. (See B)

B.—Wheat in rotation with corn and crimson clover on Field V, 1922. After corn the growth of small grains is much less influenced by the residual action of soiling crops than when following after potatoes or tobacco

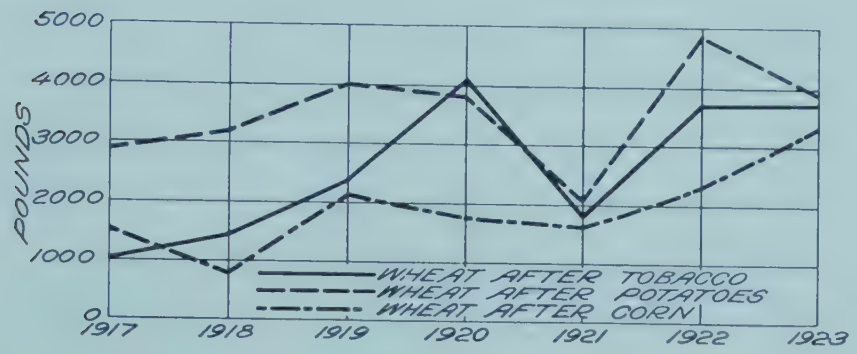

FIG. 15.—Effects of tobacco, potatoes, and corn on yield of wheat when soiling crops are in the rotation. In the early years of the test potatoes showed a markedly beneficial effect as compared with tobacco, but in subsequent years the difference between the effects of tobacco and potatoes have been much smaller. Corn has shown a depressing action throughout the test

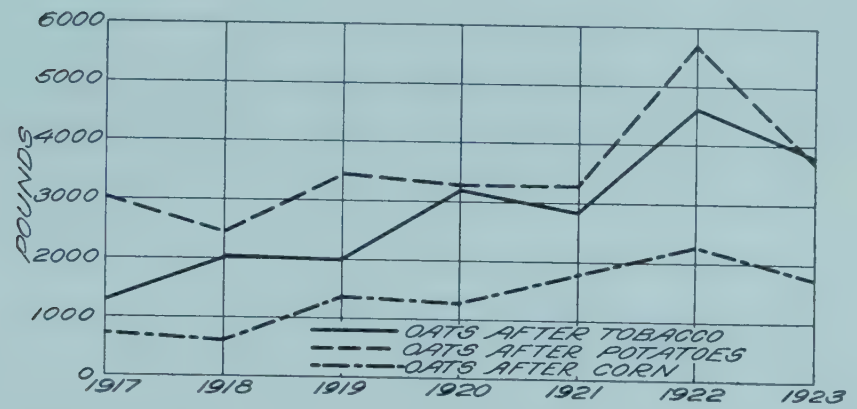

FIG. 16.—Effects of tobacco, potatoes, and corn on yield of oats when soiling crops are in the rotation. The outstanding feature is the marked upward trend in yield of oats after tobacco, the yield in later years of the test almost equaling that after potatoes; the progressive gain after corn from the residual effects of the soiling crops is comparatively small

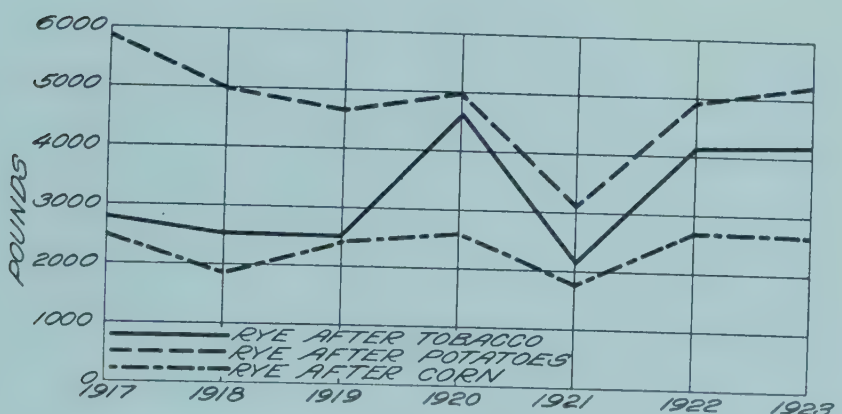

FIG. 17.—Effects of tobacco, potatoes, and corn on yield of rye when soiling crops are in the rotation. In comparison with corn, potatoes have shown a strikingly beneficial effect on the rye. This was true also of the potatoes in comparison with tobacco in the early years of the test, while, more recently, the growth of rye after tobacco has approached more nearly that after potatoes

A.—Comparative effects of roots and tops of potatoes on growth of tobacco plants in pots. At right, effect of potato roots placed in the soil and, at left, effect of tops of potatoes. Pot in center is a control. It will be seen that the roots seem to depress growth, while the tops apparently promote growth of tobacco. Similar results were obtained with tops and roots of vetch

B —Comparative effects of tops and roots of hairy vetch on growth of tobacco in the field. Row in center is a control to which no vetch material was added. Row to left of center received tops of fresh vetch plants, and row to right of center received roots of vetch plants applied to the soil about seven weeks before transplanting the tobacco. A beneficial action is shown by the tops but none by the roots

tobacco plants, the size of the leaves, and quantity of water transpired, but these data need not be given here. As shown in Plate 7, A, the roots of potatoes retarded the growth of tobacco, while the tops somewhat increased the rate of growth. The same results were obtained with the roots and tops of hairy vetch. Corn roots retarded growth somewhat, while no harmful effect was observed with tobacco roots. The tops and roots of vetch used in the tests were from fresh plants while the other plant material was from the growth of the preceding summer.

A field test was made on the experimental farm with fresh tops and roots of hairy vetch and crimson clover. The land used had been cropped to tobacco for several years. The plant material was applied in the row at the rate of two tons green weight per acre. The material was applied May 7, and the tobacco was set June 26. No fertilizer was used. Alternate rows were used as controls. With both the vetch and clover the tops gave increased growth, while the vetch roots retarded growth and the clover roots seemed to have no decided effect (pl. 7, B). These preliminary tests seem to indicate that the injurious effects of certain crops on tobacco are due to the roots. If this be true, it would seem to present new problems in the matter of supplying desirable forms of organic matter to the soil and at the same time of insuring adequate aeration.

CONCLUSIONS

Fairly extensive field experiments have been in progress in southern Maryland for several years to determine whether legumes and other soil-improving crops, combined with commercial fertilizers, can be used successfully in applying intensive methods to tobacco culture; and to study the comparative effects of various crops on those which follow in rotation, with special reference to tobacco. The soil used is the fine sandy loam type of the Collington series. The results to date do not support the view which has been often advanced that tobacco is especially injurious to the general productiveness of the soil. On the other hand, it appears that the tobacco plant is particularly sensitive to the effects of preceding crops, and attempts to apply intensive methods, as turning under soil-improving crops freely or applying large quantities of manures and fertilizers, are likely to fail. The growth of the tobacco plant may be seriously retarded as a result of the effects of preceding crops of tobacco itself or of various other plants. Under the intensive methods in question, reduced yields of tobacco do not involve a reduction in the general productiveness of the soil.

In special cropping tests with tobacco, moderately fertilized, use of hairy vetch, crimson clover, and rye as soiling crops in continuous tobacco culture has given rather large increases in yield of tobacco during the first few years of the tests. In later years, however, the yields of tobacco have been very variable, depending largely on weather conditions. In dry years the yield has been rather good, while in wet years it has been less than that of the control plot, and the quality of the leaf has been poor. There is a tendency toward very uneven growth, but both large and small plants contain a high percentage of nitrogen. Much the same results have been obtained with crimson clover and cowpeas used as soiling crops for tobacco in a two-year rotation with wheat. For the period of the tests the general trend in yield of tobacco since the initial increase from the soiling crops has been downward. In a three-year rotation of tobacco, wheat, and red clover, both the yield and the quality of the tobacco have been more stable and have averaged considerably higher than those of the control plot. It is possible, however, that these results are due largely to the fact that the land is substantially in a resting condition for the greater portion of the rotation period rather than to any specifically favorable influence of the red clover. In any event, it is a striking fact that none of the cropping systems tested have given results with tobacco equal to those obtained on rested land, that is, land occupied for a period of years by adventitious vegetation. Leaving out of account instances of infestation with Thielavia root-rot, at the present time the only system of soil management known to be effective in restoring to normal the yield of tobacco which has declined under intensive methods is the simple expedient of allowing the soil to remain idle for a period of years. It appears that while limited use of soil-improving crops under favorable circumstances may give profitable returns with tobacco, conditions may easily arise where their use would be positively detrimental. In the present series of tests, use of soil-improving crops combined with liming but without use of commercial fertilizer has soon resulted in practical failure of the tobacco crop.

The comparative effects of the three crops, tobacco, potatoes, and corn, on succeeding crops have been studied by growing each of these three crops in (1) continuous culture and in alternation with the other two; (2) rotation with each of the small grain crops, wheat, oats, and rye; and (3) rotation with each of the three small grain crops, including hairy vetch, crimson clover, cowpeas, soy beans, and a mixture of grasses as soiling crops in the rotations. Except where the soiling crops were used, four different fertilizer treatments have been used in each system of cropping. Although the potato crop removes from the soil only small quantities of plant nutrients, while corn removes much larger quantities, tobacco for the most part has resembled potatoes rather than corn in its effects on succeeding crops. In continuous culture, tobacco has shown a tendency to decline in yield, but more recently the growth after corn has been poorer than in continuous culture. Potatoes have given best yields after tobacco and poorest yields after corn. The corn crop has not been greatly affected by preceding crops. In comparison with tobacco and potatoes, the corn crop has decidedly reduced the yields of succeeding crops of wheat and oats, and, to a lesser extent, rye. In earlier years, the growth of small grains after potatoes was considerably better than after tobacco, but this difference has not been fully maintained. Addition of legumes to the cropping system has intensified rather than overcome the differences in effects of tobacco, potatoes, and corn on the small grains. The effects of vetch crimson clover, and cowpeas on tobacco have been the same as noted in the previous paragraph. Soy beans have given especially poor results with tobacco and potatoes. Grass as a soiling crop has markedly reduced the yield of tobacco and potatoes, and to a lesser extent, the yield of corn. Neither tobacco nor potatoes have been able to effectively utilize the nitrogen of the legumes. Corn and the small grains, on the other hand, have been greatly benefited by the legumes, readily taking advantage of the nitrogen furnished by them.

The results of these cropping tests furnish a group of crop effects which are not wholly explainable either on the basis of the plant-food theory or of parasitic disease. These crop effects are of a transitory character, in so far as they are toxic or injurious, and are markedly influenced by the character of the soil and the weather conditions. They are also influenced to some extent by fertilizers and lime. Preliminary field and pot tests with tobacco indicate that the injurious effects of preceding crop plants come mostly from the roots rather than the tops of these plants. If these results are confirmed, they would seem to suggest new problems in the matter of most effectively supplying the soil with organic matter.

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SOME EFFECTS OF SEASONAL CONDITIONS UPON THE CHEMICAL COMPOSITION OF AMERICAN GRAPE JUICES [1]

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PURPOSE OF THE WORK

The studies here reported were undertaken for the purpose of obtaining information as to the effects of variation in climatic conditions over a series of years upon the chemical composition of some of the more important varieties of American grapes, when grown together under known and controlled conditions of cultivation, pruning, and fertilization upon a soil of known physical character and chemical composition.

While a considerable number of chemical analyses, covering most of the older and more widely known commercial varieties of grapes, may be found in the literature, few of them are accompanied by any data on the cultural treatment received by the vines or the climatic conditions under which the analytical material was grown. Before the results of chemical analyses can be employed to formulate the definition in chemical terms of desirable dessert quality or to guide the breeder in selection of more desirable varieties, the extent to which annual variations in environmental conditions affect chemical composition must be definitely known.

No systematic attempt, so far as is known to the writer, has ever been made to secure uniformity in all cultural treatments over a series of years for a large number of varieties growing side by side and to determine the nature and extent of the fluctuations in chemical composition of the fruit resulting from the variations in environmental conditions encountered during the production of a number of crops. The writer knows of no data in the literature indicating the extent to which such seasonal conditions as drought, excessive rainfall, minimum sunshine during the growing period, or other departures from normal in environmental conditions affect the chemical composition of the crop.

Investigations of methods for the more effective utilization of some of the varieties of grapes grown throughout the Central and Eastern States were undertaken by the Office of Horticultural Investigations of the Bureau of Plant Industry, United States Department of Agriculture, in 1918. These investigations were made on account of the fact that there is sometimes a considerable surplus of varieties grown for dessert use and the products from varieties formerly grown extensively for wine making have not yet found a profitable place in legitimate markets. One outlet for a considerable part of the grape surplus is in the manufacture of unfermented grape juice, where there is a demand in excess of the supply. To satisfactorily meet this demand, the juices must not only be acceptable in beverage quality but must be standardized and uniform in character. Relatively few grapes produce juices having such a balance between sugar, acid, and astringent constituents as to be wholly satisfactory for making unfermented beverages; no other grape has seriously rivaled the Concord, which possesses such a balance, as a source of juice. Successful additions to the beverage-juice supply are scarcely to be looked for through the making of single-variety juices, but the blending of juices of two or more varieties offers wide possibilities for the making of products of any desired character.

Before such work can proceed in any other than purely haphazard, cut-and-try fashion, accurate information as to the chemical composition of the various new materials must be in hand. In order not to be misleading, such information must furnish an idea as to the extent and nature of the variations in chemical composition which are likely to occur in the juices of given varieties from year to year as a result of varying seasonal conditions. Such

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a background of information as to chemical composition would indicate also the other uses to which the various varieties are adapted. This paper presents the results of an attempt to secure such a background.

THE EXPERIMENT VINEYARD

Location

The Office of Horticultural Investigations of the Bureau of Plant Industry established an experiment vineyard of selected varieties at Vineland, N. J., in 1908, which was the source of the material used in the work.

Vineland is located near the northern boundary of Cumberland County, which borders upon Delaware Bay at the extreme southern tip of New Jersey. The experiment vineyard is located about 1¼ miles southeast of the town limits. The Millville area, within which the vineyard lies, has been described in detail by the Bureau of Soils, United States Department of Agriculture, (*20*)[2] and the chemistry of the soils of the area has been studied by the New Jersey Agricultural Experiment Station (*15*). The description of the locality given here is summarized from the publications just referred to.

The area is on the Coastal Plain, and is characterized as a whole by its flatness and lack of relief. The region adjacent to Vineland has a number of low, flattened ridges rising from 10 to 30 feet above the general surface, which is 109 feet above sea level at Vineland. The soils of the whole area are of comparatively recent and largely marine origin, the Cohansey sand formation overlying most of the area, and consist for the most part of sand and gravel with a small percentage of clay. In the locality in which Vineland is situated the soil is classified as Sassafras gravelly sandy loam. The surface soil is a light brown loamy sand to sandy loam, 6 to 8 inches in depth, beneath which is a yellow or yellowish-red sandy loam 4 to 8 inches in depth. The subsoil is a dull red or reddish-yellow sandy clay with some grave and coarse sand, which are in greater proportion with greater depth. White and yellow quartz gravel one-eighth to three-fourths inch in diameter occur in varying proportions throughout both surface soil and subsoil. While somewhat deficient in plant food and requiring addition of lime, as indicated by the analyses in Tables I and II, the soils have good drainage, become warm early in the spring, and are easy to cultivate. About 50 per cent of the Sassafras gravelly sandy loam in the area has been cleared and is devoted to the cultivation of vegetables and fruits. Sweet potatoes, peppers, tomatoes, sweet corn, strawberries, raspberries, Lima beans, and peas are the principal truck crops. The area immediately adjacent to Vineland has become a rather intensive peach-growing district, and the planting of apples is increasing. The tree fruits have to a considerable extent displaced grapes, which were formerly much grown but which gradually became unprofitable on account of improper management. Where effective methods of controlling disease and maintaining soil fertility have been adopted the vineyards continue to yield profitable crops.

TABLE I.—*Composition of Sassafras loamy sand and Sassafras gravelly sandy loam, Vineland, N. J.*

Soil type and location	Depth sampled	Percentages of—					
		Nitrogen (N)	Phosphoric acid (P_2O_5)	Potash (K_2O)	Lime (CaO)	Magnesia (MgO)	Carbon (C)
	Inches						
Sassafras loamy sand 1 mile east of Vineland, at Spring Road and Landis Avenue	0 to 9	0.050	0.083	0.373			0.750
Subsoil, same	9 to 36	.023	.043	.947			.215
Sassafras gravelly sandy loam, near Vineland	0 to 8	.089	.083	.685	0.315	0.290	1.589
Subsoil, same	8 to 34	.028	.035	.685	.210	.319	.309
Sassafras loamy sand at Norma, 3½ west of Vineland	0 to 10	.053	.077	.338	.105	.152	.764
Subsoil, same	10 to 36	.021	.034	.382	.203	.232	.089

[2] Reference is made by number (italic) to "Literature cited," p. 1175.

TABLE II.—*Pounds of plant food per acre in upper 6⅔ inches (approximately 2,000,000 pounds of soil) in Vineland, N. J. soils*

Soil type and location	Depth sampled	Nitrogen (N)	Phosphoric acid (P_2O_5)	Potash (K_2O)	Lime (CaO)	Magnesia (MgO)	Carbon (C)	Lime needed per 2,000,000 pounds of soil
Sassafras loamy sand 1 mile east of Vineland, at Spring Road and Landis Avenue	*Inches* 0 to 9	1,000	1,660	7,460	----------	----------	15,000	800
Subsoil, same	9 to 36	460	860	18,940	----------	----------	4,300	----------
Sassafras gravelly sandy loam, near Vineland	0 to 8	1,780	1,660	13,700	6,300	5,800	31,780	3,200
Subsoil, same	8 to 34	560	700	13,700	4,200	6,380	6,180	----------
Sassafras loamy sand at Norma, 3½ miles west of Vineland	0 to 10	1,060	1,540	6,760	2,100	3,040	15,280	2,000
Subsoil, same	10 to 36	420	680	7,640	4,060	4,640	1,780	----------

Data for Tables I and II taken from Blair, A. W., and McLean, H. C. (*15*).

The average mean winter temperature at Vineland is 33°, for spring 51.1°, for summer 73.9°, for autumn 55.4°, the mean for the year being 53.3°. The mean spring and summer temperatures are higher, the mean fall and winter temperatures lower than those at coast points such as Atlantic City and Cape May city, while the maximum and minimum temperatures show a somewhat wider range than is the case nearer the ocean. The winters are much milder than those of northern New Jersey but are accompanied by high humidity, which often renders the cold very penetrating. There is usually very little snow, and it seldom remains long on the ground. The maximum temperatures for the year usually occur in July at Vineland, and are frequently accompanied by high humidity which makes them quite oppressive. July and August are the months of greatest rainfall, the total annual precipitation for the 50-year period averaging 46.59 inches. The growing season is considerably shorter than at coast points, averaging 185 days in length. The average date of the last spring frost is April 18; that of the first fall frost October 20.

PLAN AND HISTORY

The experiment vineyard from which material for this investigation was obtained was established by the Bureau of Plant Industry in 1908, when a block of 10 acres was planted on the property of the Vineland Grape Juice Co. The experiments for which the planting was originally made were continued for 11 years. They included a study to determine the best varieties for the region, a study to determine the best methods of pruning and training for the varieties under trial, and tests of cover crops, clean culture, fertilizers, and spray mixtures. At the end of 1919 these experiments were terminated and the care of the vineyard was continued by the Vineland Training School for the Feebleminded, which had acquired the property. In 1920 the tract was purchased by Frank C. Bray.[3] The varietal collection was kept intact and the same methods of pruning and spraying employed prior to 1919 were continued, while the general methods of fertilization, cover cropping, and cultivation employed were those in use in the best commercial vineyards of the region. At the time the collection of material for analyses was begun in 1919 the vines were, for the most part, 11 years old, the principal planting having been made in 1908, with a few minor additions in 1909 and 1910. They had received the best of care and had been in excellent condition up to the year 1917. A very severe hailstorm on June 11, 1916, did considerable damage to vines and fruit. A rather serious attack of rootworm (*Phidia viticida*) in the year 1917 necessitated vigorous control measures during the following season and resulted in the death of a number of vines, while a few of the less vigorous varieties required several years to recover from the attack. On August 14, 1918, a very severe wind and rain storm, at a time when the ground was thoroughly saturated, did

[3] The writer desires to express his obligations to the authorities of the Vineland Training School and to Mr. Bray for the opportunity to obtain samples of fruit for use in this investigation, and for the facilities afforded him for the work.

considerable damage by tearing down trellises and breaking vines. With the exception of about a dozen varieties which showed more or less injury from these causes, the vineyard was in excellent condition when the collection of samples was begun in 1919.

The varietal plantings from which the fruit used in the studies here reported was taken occupy a rectangular block of 10 acres, situated on the crown of a low, flattened ridge which runs northeast and southwest. The plot is not perfectly level but is on gentle southern and northern slopes, with the western edge sloping slightly toward the northwest. The difference in elevation is not more than 5 or 6 feet, except at the extreme northwestern corner. The elevation above sea level at the center of the plot is 118.5 feet. The rows of vines run northeast to southwest.

The total number of varieties in the vineyard is 67. The main planting consists of 144 vines each of the following 27 varieties: Brilliant, Campbell Early, Catawba, Concord, Cynthiana, Delaware, Diana, Dutchess, Elvira, Goethe, Herbemont, Herbert, Ives, Lenoir, Lindley, Montefiore, Moore Early, Niagara, Noah, Norton, Pocklington, Salem, Vergennes, Winchell, Wilder, Woodruff, and Worden; with 72 vines each of the 5 varieties: Clevener, Diogenes, Franklin, King Philip, and Martha. As each row in the vineyard contained 72 vines, each variety of the group of 27 varieties first named occupied two full rows, and each variety of the last-named group occupied one row. In order to avoid any differences due to the different conditions on the north and south slopes, the planting of each variety was distributed equally between north and south sides of the vineyard.

In addition to the principal plantings, a plot lying on the eastern side of the main block had been planted to 10 vines each of the following 31 varieties: Agawam, Barry, Berckmans, Brighton, Canada, Centennial, Colerain, Champion, Diamond, Eaton, Early Daisy, Early Victor, Eumelan, Gaertner, Isabella, Iona, Jefferson, Hartford, Lampasas, Lucile, Lady, Massasoit, Merrimac, Missouri Riesling, Nectar, Perkins, Rommel, Ulster, and three varieties designated in the records as "Studley No. 2," "Seibel Hybrid No. 1," and "Seibel Hybrid No. 2." In addition, there were in the original plantings four to six vines each of Beta, Dakota, "Dutchess seedling," Downing, Geyer, Husmann, Rebecca, "Viticultural No. 3405," and "Viticultural No. 3506." Of these, only "Dutchess seedling" and Rebecca bore fruit, and these only in 1922 and 1923. At the time this work was begun in 1918 no vines of Beta remained living, and the vines of Downing, Geyer, and Husmann never bore more than an occasional cluster of fruit. The varieties designated as "Viticultural No. 3405" and "Viticultural No. 3406" bore occasional clusters of small, worthless fruit and there was an entire failure to mature. The block of vines designated as "Studley No. 2" contained two varieties neither of which was Studley No. 2 and neither of which has been positively identified. Consequently none of these varieties appears in the reports of analyses.

In order to test various types and amounts of fertilizer and different methods of pruning and training, the vineyard had been divided, at the time of planting, at right angles to the rows of vines, into 12 plots each 6 vines in width. These 6 vines were pruned and trained by different methods, the first by low renewal, the second by four-arm renewal, the third by high renewal, the fourth by the fan system, and the fifth and sixth by the Munson system. A thirteenth plot contained all the small plantings of 10 vines of a variety with a few vines each of miscellaneous varieties forming a border. Each of the larger plantings extended across the other 12 plots, hence had equal numbers of vines trained by each of the systems except the Munson, which had twice as many as any of the others.

The effects of injury from the attack by rootworm in 1917 and 1918 were evident in a number of varieties for several years in the form of slow growth, deficient vigor, and light yields. The varieties which were obviously not normal in condition in 1918 were Brilliant, Campbell Early, Catawba, Cynthiana, Colerain, Early Victor, Iona, Jefferson, Lady, Lenoir, Nectar, Norton, Pocklington, and Woodruff. By careful handling and restricting the crop they were allowed to carry, Catawba, Cynthiana, Brilliant, Nectar, Colerain, Pocklington, Norton, and Jefferson were brought back to normal vigor and fruitfulness in 1919. A considerable number of vines of Campbell Early and Iona had died or been cut back to the ground so that only a part of the original vines were bearing; those not cut back made only partial recovery, but the new growth from old stumps was normal. Lady, Early Victor, Nectar, and Woodruff remained below normal in vigor of growth and size of crop throughout the experiments, Woodruff

especially ripening very irregularly, dropping its foliage badly, and failing to color a portion of its fruit. Lenoir bore rather light crops in 1919 and 1920, but had fully recovered by 1921.

In view of these conditions, the varieties may be divided into two groups, one consisting of 49 varieties which were normally vigorous in vine and fruit and which bore annual crops of fair size, ripening their fruit uniformly. (The data for these varieties are presented in Table III.) The other 16 varieties include all those which for any reason failed to fruit annually and in fair amount through the period of the experiment. The reasons for the failures were various, but some of them were poor adaptation to the environment, susceptibility to insect or fungus injury, to scorching of foliage by spray, partial sterility, or shy bearing. Analyses of these, with summaries of the field notes as to their behavior, are given in Table IV. All comparative discussion is based upon the results with the 49 normal annual-bearing varieties of Table III.

BOTANICAL RELATIONSHIPS OF THE VARIETIES

Some of the varieties used were purebred and some were hybrids. While horticulturists are by no means unanimously agreed as to the parentage of several of the varieties, the following table is believed to represent the majority of opinion. The statements of Hedrick (*29*, *30*) have been followed in the main, supplemented by those of Munson (*49*) and the Bushberg catalogue (*16*).

1. *Purebred Vitis labrusca:* [4]
   - Champion.
   - Colerain (white seedling of Concord).
   - Concord.
   - Early Daisy (seedling of Hartford).
   - Eaton (seedling of Concord).
   - Hartford.
   - Ives.[5]
   - Lady (seedling of Concord).
   - Lucile (seedling of Wyoming).
   - Moore Early (seedling of Concord).
   - Pocklington (seedling of Concord).
   - Vergennes.
   - Worden (seedling of Concord).
2. *Labrusca-vinifera hybrids:*
   - Agawam (Labrusca × Black Hamburg).
   - Barry (Labrusca × Black Hamburg).
   - Brighton (Concord × Diana Hamburg).
   - Campbell Early (Moore Early × Labrusca × Vinifera).
   - Catawba.
   - Diamond (Concord × Iona).
   - Diana (seedling of Catawba).
   - Gaertner (Labrusca × White Chasselas).
   - Goethe (Labrusca × Black Hamburg).
   - Herbert (Labrusca × Black Hamburg).
   - Iona (seedling of Diana.[6])
   - Isabella.
   - Jefferson (Concord × Iona).
   - Lindley (Labrusca × White Chasselas).
   - Martha (seedling of Concord).
   - Massasoit (Labrusca × Black Hamburg).
   - Merrimac (Labrusca × Black Hamburg).
   - Niagara (Concord × Cassady).
   - Perkins.
   - Rebecca.
   - Salem (Labrusca × Black Hamburg).
   - Ulster.
   - Wilder (Labrusca × Black Hamburg).
   - Woodruff (Concord × Catawba).
3. *Labrusca-aestivalis* hybrids:
   - Cynthiana.[7]
   - Norton.[7]
4. *Labrusca-aestivalis-vinifera* hybrids:
   - Centennial (Labrusca × Eumelan seedling).
   - Eumelan.[8]
   - Winchell.
5. *Labrusca-bourquiniana* hybrids:
   - Early Victor (Delaware × Hartford) (?)
6. *Labrusca-vinifera-bourquiniana* hybrids:
   - Brilliant (Lindley × Delaware).
   - Delaware.
   - Dutchess (Concord seedling × Walter or Delaware).
7. *Labrusca-vulpina* hybrids:
   - Clevener.
   - Clinton.
   - Dakota (Concord × vulpina ?).
   - Diogenes.
   - Elvira (seedling of Taylor).
   - Franklin.
   - Missouri Riesling (probably seedling of Taylor).
   - Montefiore (Taylor × Ives).
   - Noah (seedling of Taylor).
8. *Labrusca-vulpina-vinifera* hybrids:
   - Canada (Clinton × Vinifera).
   - King Philip.
   - Rommel (Elvira × Triumph).
9. *Labrusca-vulpina-bourquiniana* hybrids:
   - Berckmans (Delaware × Clinton).
10. *Purebred Vitis bourquiniana:*
    - Herbemont.
    - Lenoir.

This collection of grapes is characterized by the absence of purebred varieties of species other than Vitis labrusca and bourquiniana and by the presence of a strain of labrusca in all the hybrids. In consequence the results do not adequately represent the range of varieties which are at present available to growers through the work of breeders who have employed other species as parents. These varieties include practically all the older and more widely known varieties which were grown in the Central and Eastern States 15 years ago, hence give information in regard to nearly all varieties which have attained commercial importance in that area.

[4] Tukey (*59*) is inclined to the belief that Vitis vinifera blood may in reality be much more generally present in American varieties than has hitherto been considered to be the case. He states some evidence that Concord and its seedlings have Vinifera in their ancestry, and regards Vergennes as undoubtedly containing a strain of Vinifera.

[5] Ives is considered by Munson and the authors of the Bushberg catalogue as pure Labrusca, while Hedrick considers it a Labrusca-aestivalis hybrid.

[6] This is Hedrick's statement (*30*, *p. 390*), the Bushberg catalogue (*16*, *p. 111*) states that Iona is a seedling of Catawba.

[7] The Bushberg catalogue lists both Cynthiana and Norton as pure Aestivalis, to which Munson agrees.

[8] The Bushberg catalogue (*16*, *p. 99*), calls Eumelan an Aestivalis, remarking that it was designated as Labrusca in the first edition by a typographical error and that the error has been widely copied.

THE METHODS EMPLOYED

Collection and Preparation of Samples

In any work of this character the value of the results hinges wholly upon the securing of samples for analysis which are truly representative of the crop as a whole. The method of collecting samples here employed was purposely chosen to eliminate the errors inherent in the selection of small samples of fruit which have been pointed out by Denny (*19*) and by Haynes and Judd (*28*) and was made easier by the fact that considerable quantities of the juice of each variety were desired for other experimental purposes. In the case of the varieties of which there were only 10 vines, the entire crop of the 10 vines was harvested, thus making a sample of 30 to 75 pounds. In the case of those varieties of which there were 72 or 144 vines each, the entire crop could not be used, and the owners usually picked the fruit of a given variety for sale about 6 to 10 days before it was judged to be fully ripe. Consequently the entire planting of a variety was examined and a number of vines standing consecutively in the row and including equal numbers of vines trained by each of the methods of pruning employed and ranging from 15 to 40 in number were reserved for use in this work and the picking was postponed until the crop was fully ripe. The block of vines so chosen was thus as truly representative of the whole planting of the variety, in appearance and condition of vines and size of crop, as it could be made, and the entire crop from the block was harvested together, the pickers being instructed to leave no fruit on the vines. The samples so obtained ranged from 50 to 300 pounds in weight, usually averaging about 150 pounds. It is believed that these samples were as free from the effects of conscious or unconscious selection and as truly representative of the whole product of the vines as could be.

As rapidly as picked, the fruit was conveyed to a building near the vineyard and pressed. Unless otherwise noted, all samples were cold pressed, i. e., pressed without previous heating. A large-size hand cider press was used, the grater being adjusted so as to burst the individual grapes without crushing the seeds. Racks and cloths were substituted for the slatted device usually supplied with such presses. A sufficient number of cloths were provided to permit the washing of each cloth after it was once used and drying it before it was used again, and the press and racks were thoroughly cleaned and washed after the pressing of each variety was completed. About 50 to 65 pounds of fruit could be handled at one pressing. In order to accomplish an extraction of juice comparable with that obtained by the smaller hydraulic presses, a 6-foot lever was used and the screw was forced down as far as two strong men could turn it. After draining had stopped, the press was opened, the cakes were broken up and returned to the cloths and the pressing repeated. That the extraction of juice was comparable to that obtained in ordinary commercial practice by the use of a hydraulic press is evidenced by the yields of juice obtained, which ranged from 6.9 to 8 gallons per 100 pounds of fruit.

Juices expressed in this manner will differ appreciably in composition from those obtained by pressing small samples of fruit through muslin by hand, though some workers have assumed that such hand-pressed samples accurately represent the composition of the crop. The writer has repeatedly made analyses of samples made from the same lot of grapes, by pressing in the press here used, and by crushing the fruit, placing it in a strong cloth, and squeezing with the hands until no more juice could be expressed. In every case a less complete extraction of juice was obtained by hand pressing, as shown by the weight of the residues. Hand-pressed samples show considerably lower acid, higher sugar, and higher total solids than do those made with a press, and these differences, superposed upon the error which usually occurs in taking small samples, give analytical results materially higher for sugar and solids than the true ones.

The juice was received as it drained from the press into enamel-lined pails of a size sufficient to contain the juice from one pressing. As soon as the pressing of a variety was complete the juice was carried into the workroom and sampled. If the juice from a variety occupied only one container, it was thoroughly stirred while portions for the various determinations were pipetted into beakers. If the lot of fruit was of such size that the juice filled two or more containers, an equal amount was measured from each, after thorough stirring, into another vessel, and the samples for analysis were taken from this vessel. Thorough agitation during the taking of samples is by no means superfluous, as many juices throw down an abundant flocculent precipitate immediately after pressing.

In the first two years of the work, 1919 and 1920, the determinations made in the field were confined to acid content, specific gravity, and sugar content by the Brix scale saccharimeter. Beginning in 1921, determinations of total astringency and nontannin astringency were also made. All the determinations upon any given sample of juice were invariably completed within 30 minutes after pressing was completed. The remainder of the juice was then placed, after again stirring to secure uniformity, in quart bottles, half-gallon glass jugs, or in fruit jars of the Lightning Seal type, sealed, numbered serially for identification, and pasteurized by submerging in cold water, bringing to 80° C. for 5–15 minutes, and cooling slowly out of drafts. Analytical work upon the juices so prepared had to be postponed until the winter of 1921–22, the juices in the meantime having been stored in the original containers after shipment to Washington from Vineland. The desirability of securing additional data upon the freshly pressed juices for comparison with those subsequently to be obtained from the same juices after pasteurization became apparent as soon as the analytical work was well under way. In consequence, the preparation of samples in 1922 and 1923, in addition to samples pasteurized as in the previous years, included the preparation of duplicate samples which were preserved with bichloride of mercury, without heating, for sugar determinations. The bulk of the juice was pasteurized as in previous years.

The methods employed in the field determinations were essentially those of the Association of Official Agricultural Chemists (*10*), with the following modifications:

Acidity.—Twenty-five c. c. of juice was pipetted into a 400 c. c. beaker and made up to 250 c. c. with water, placed in such a position before a shuttered window that a beam of sunlight illuminated it from the side opposite the operator, and titrated with N/10 sodium hydroxide against phenolphthalein as indicator. By careful attention to the series of color changes occurring in the solution it was possible to be certain of the end point, even in the deeply colored juices, but the readings obtained upon such juices were checked by the use of phenolphthalein powder and a spot plate. While it is recognized that titration of a liquid such as grape juice against phenolphthalein gives high results through the inclusion of some gallotannic acid, as Gore (*24*) has shown, no other method capable of being used under field conditions gives more accurate or satisfactory results. Since the method has been used in practically all published determinations upon grape juices, it serves the purpose of the present work by yielding results which permit direct comparison.

Specific gravity.—A precision hydrometer, reading to the third decimal place, was employed.

Sugar content by Brix scale.—The value of data obtained by the use of an instrument intended for determinations upon relatively pure sucrose solutions when juices containing mainly or wholly reducing sugars and widely varying amount of nonsugar solids were to be tested seemed questionable at the outset, but as it was impossible to keep the analytical work abreast of the field work spindle readings were made. Alwood (*5*) has discussed the comparative accuracy of Oeschle, Balling, Baumé, specific gravity, and Brix spindles, reaching the conclusion that Brix determinations with a specially made type of spindle are sufficiently accurate for comparative purposes in work upon grape and apple juices if an average of the nonsugar solids of the different varieties is allowed in each case, notwithstanding the fact that such nonsugar solids range from 1.5 to 4 per cent in different varieties. Thompson and Whittier (*56*) take issue with this conclusion, stating that—

> methods employing specific gravity determinations (as by the use of the Brix spindle) as a basis for calculating the total sugars or even the solids, are very questionable on unknown solutions. In order to use such a method on a fruit juice, it is first necessary to accomplish an accurate analysis upon each fruit juice and even upon each variety of the same fruit before a spindle can be used with any degree of accuracy for determining the total sugars.

Brix precision hydrometers, calibrated to 0.1 per cent of sugar at 17.5° C., of the type recommended by Alwood, were used. As the temperatures at which the field readings had to be made were those of an open shed and varied from 6.5 to 32.7° during the work, it was not expected that the corrected readings would give more than approximations of the sugar content as determined by analysis. As soon as the analytical work was under way it became evident that the readings were without much value either for comparison of varieties with one another or for comparing the juices of the same variety in different years. The nonsugar solids of the varieties studied range from 0.57 to 4.5 per cent, and the amount in a given variety fluctuates very considerably from year to year. The occasional presence of varying amounts of cane sugar in grape juice

also contributes to inaccuracy in spindle readings. Consequently such readings were discontinued after 1921, and none of the figures either for Brix or specific gravity are published.

TOTAL ASTRINGENCY.—Determinations were made by the Loewenthal-Proctor method, employing 25 c. c. of juice diluted to 750 c. c. and titrated in presence of indigo-carmine with N/20 $KMnO_4$. Astringent nontannins were determined after precipitation of the tannins by gelatin-salt solution, shaking with kaolin, and filtering. The results are expressed in grams by the use of the conventional factor (1 c. c. N/20 $KMnO_4$ =0.0020785 gram tannin), although it is recognized that for astringent nontannins these figures do not express absolute values and are servicable only for purposes of comparison.

PRESERVATION OF SAMPLES FOR ANALYSIS

In 1919, 1920, and 1921 the samples intended for analysis were duplicate quart bottles which were filled from the thoroughly stirred bulk sample, sealed, numbered, and pasteurized in the same manner as the remainder of the juice. The analytical work upon these samples had to be postponed until late in the autumn of 1921. In the meantime work upon the nature and amount of change in chemical composition produced in apple juices by pasteurization was in progress in the laboratory and the results obtained made it seem desirable to extend the study to grape juices. The fact that sucrose had been found in considerable quantity in the pasteurized juice of a number of varieties was an additional reason for securing data on unpasteurized juices. Samples of all varieties were preserved in 1922 and 1923 by the use of bichloride of mercury. Two half-pint bottles were filled with the juice, after thorough stirring to secure uniformity, 0.25 gram of powdered c. p. bichloride of mercury was added to each bottle, the bottles were vigorously shaken to bring the mercury into solution, sealed, and numbered. Two pint or quart bottles were filled with the same precautions to secure representative samples, sealed, and pasteurized to serve for analysis of the pasteurized juice, as in previous years.

ANALYSIS OF SAMPLES OF JUICE

Analysis of the samples of 1919–1921 which had been shipped to Washington and stored at room temperature in a dark room, protected from freezing, in the laboratory, was begun late in the autumn of 1921. The samples were analyzed in the order of age, the 1919 samples being taken first, then those of 1920. By the time these analyses were completed the work was interrupted by the harvest of 1922. The sugar determinations on the 1922 samples preserved with bichloride of mercury were begun a few days after the receipt of the samples at the laboratory and were completed within three weeks, taking the samples in the order in which they had been prepared so that none of them stood longer than four weeks before being handled. Upon completing the work with these, the analysis of the pasteurized samples of 1921 and 1922 was taken up and completed prior to the 1923 harvest. On receipt of the 1923 samples at the laboratory, sugar determinations were at once made upon those preserved with bichloride of mercury, followed by analysis of the pasteurized samples of the same crop. So, the pasteurized samples of the crop of 1919 had been in storage a little more than three years when analyzed, those of 1920 about two and one-half years, those of 1921 about fifteen months, those of 1922 and 1923 five to seven months each, when the analyses were made. All samples were stored together, for the most part in the original shipping crates, in a dark room in which the temperatures ranged between 50 and 95° F. until taken out for analysis.

A question which naturally arises, namely, whether the analytical data obtained from juices pasteurized and stored for a period of three years is comparable with that from samples stored only a few months, can be definitely answered in the affirmative. The coagulation and precipitation of pectins, tannins, and other constituents which result from the heating of a juice in pasteurization continues for a limited time after the juice has cooled and been placed in storage, but comes to an end in the course of six to twelve months. Repeated analyses of samples of the juices here used, as well as the results of extensive studies of the changes induced by pasteurization in both apple and grape juices to be reported in detail in another publication, make it clear that a sterile pasteurized juice, stored in darkness and protected from freezing or overheating, attains a constant composition within a few months and will remain unchanged indefinitely under such conditions (*27*).

Analytical Methods

The samples preserved by addition of bichloride of mercury were used for sugar determinations only. No correction was necessary for the amount of $HgCl_2$ used (0.25 gram for 250 c. c. of juice).

Reducing sugars were determined by clarification with neutral lead acetate, removing the excess lead with potassium oxalate, and employing the Munson and Walker method, titrating the cuprous oxide with N/20 $KMnO_4$ after solution in ferric ammonium sulphate. The results are expressed as invert sugar or as invert sugar in presence of sucrose accordingly as sucrose was found to be present or absent. Total sugars were determined by inversion of a portion of the clarified solution and application of the same method. Sucrose was determined by difference of the figures before and after inversion. Duplicate determinations were run in all cases and were repeated when the amounts of $KMnO_4$ required for titration of the copper differed by more than 1 per cent.

Acidity, total astringency, and astringent nontannins were determined by the same methods employed in the field determinations.

Total solids were determined upon special samples used only for that purpose. Before being opened the bottle was shaken vigorously to bring the sediment into suspension, and duplicate 10 c. c. samples were pipetted with a wide-tipped pipette into flat-bottomed 10 cm. Petri dishes. Sufficient distilled water was added to each dish to spread the liquid in a uniform film over the bottom, the dishes were brought almost to dryness on a water bath, and the drying was completed in a vacuum oven at 90° C.

ANALYTICAL DATA

The analytical data are given in Tables III and IV.

Discussion of the Analytical Data

Table III brings together all the analytical data for 49 varieties which fruited yearly during the 5-year period while Table IV presents similar data for 16 varieties which did not fruit regularly during this time. Some of the outstanding facts shown by the figures may be discussed somewhat in detail.

SUGAR CONTENT

The range of variation in sugar content during the 5-year period shows large differences among the varieties, ranging from 1.47 per cent in Woodruff to 8.04 in Jefferson and Delaware. In two varieties the range of variation is between 1 and 2 per cent of sugar; in twenty-five it is 2 to 4 per cent; in sixteen, 4 to 6 per cent; in three, 6 to 8 per cent; and in two the variation exceeds 8 per cent. It might be assumed that a narrow range of variation in sugar content indicated perfect adaptation to the local conditions while a wide range indicated that the variety was out of its proper environment or subject to a variable factor such as disease attack. This assumption is scarcely borne out by the facts, since among the varieties showing a range of variation of 3 per cent or less are Concord, Cynthiana, Elvira, Martha, and Woodruff, while those showing a range of 6 to more than 8 per cent are Agawam, Brighton, Delaware, Eumelan, Jefferson, and Nectar. Of these, Cynthiana is the only distinctively Southern variety, Nectar is the only one of the group lacking in vegetative vigor and resistance to disease, and Woodruff is the only one which is susceptible to injury by spraying or fails to ripen its fruit uniformly and with characteristic color. The others are varieties which are well adapted to the Vineland region, as shown by their vigor and productiveness and freedom from insect or fungus attack. The explanation of the wide range of variation in the six varieties last named would appear to be fairly clear. All are midseason or late maturing at Vineland. Two years, 1919 and 1923, were exceptionally favorable for the maturing and ripening of long-season varieties, and the maximum sugar content for four of these occurred in 1923, for the other two in 1919. The maximum for three of the six varieties occurred in 1922, the least favorable year of the period. Additional evidence that these wide variations in sugar content are attributable to the variations in seasonal conditions will be brought out in the subsequent discussion.

With the exception of the six varieties just mentioned, the range of variation in sugar content is from 1 to 6 per cent. Hartmann and Tolman (*27*) analyzed samples of 104 commercial juices of Concord grapes, collected at factories during the season of 1912, 1913, and 1914, and representing the Hudson River district, the Chatauqua belt, and the Lake Erie district. These samples had sugar contents ranging from 11.41 to 17.53 per cent, a range of 6.12 per cent. Alwood and his collaborators (*2*, *3*, *6*, *7*) made analyses of a very large number of varieties of

TABLE III.—*Analytical data for 49 varieties fruiting annually, 1919 to 1923, at Vineland, N. J.*

	Year	Date picked	Reducing sugar	Cane sugar	Total sugar after inversion [a]	Acid as tartaric	Total astringency	Tannin	Non-tannin	Total solids	Acid-sugar ratio	Remarks
							Grams per liter	*Grams per liter*	*Grams per liter*			
Agawam	1919	Sept. 22	16.26	0.0	16.26	0.742					1:21.9	
	1920	Sept. 18	15.02	.0	15.02	1.237				18.04	1:12.1	
	1921	Sept. 23	15.91	.25	16.16	.862	1.451	0.614	0.813		1:18.6	Unaffected by frost.
	1922	Sept. 16	12.94	.26	13.20	.937	1.268	.258	1.010		1:14.0	
	1923	Sept. 23	18.38	1.76	20.14	.894	.880	.256	.624	21.16	1:22.5	
Barry	1919	Sept. 22	13.17	.29	13.46	.828					1:16.2	
	1920	Sept. 27	13.48	.0	13.48	1.335					1:10.1	
	1921	Sept. 19	11.28	1.81	13.09	1.350	1.623	.464	1.159	16.21	1:9.7	85 per cent of normal crop.
	1922	Sept. 22	13.88	.59	14.47	1.323	2.002	.742	1.260		1:10.9	
	1923	Sept. 26	16.47	.0	16.47	1.048	1.094	.551	.543	17.16	1:15.6	
Berckmans	1919	Sept. 30	18.00	.0	18.00	1.155					1:15.5	Ripens irregularly.
	1920	Oct. 1	17.72	.0	17.72	1.132					1:13.5	
	1921	Sept. 26	16.09	.0	16.09	1.192	.884	.288	.596	17.86	1:13.5	75 per cent of a crop.
	1922	Sept. 23	19.12	1.10	20.22	1.508	.958	.259	.699		1:12.1	
	1923	Sept. 27	15.57	.78	16.35	1.186	.684	.196	.488	16.72	1:13.8	
Brighton	1919	Sept. 22	23.08	.0	23.08	.570				24.16	1:40.5	
	1920	Sept. 20	16.96	.0	16.96	.660					1:25.7	
	1921	Sept. 24	17.20	.0	17.20	.637	1.392	.428	.964		1:27.0	40 per cent of a crop.
	1922	Sept. 19	18.62	.0	18.62	.758	1.657	.733	.924		1:23.8	
	1923	Sept. 18	21.84	.54	22.38	.737	1.051	.453	.598	23.66	1:30.3	
Brilliant	1919	Sept. 26	17.62	.0	17.62	.645				20.08	1:27.3	
	1920	Sept. 25	15.80	.0	15.80	.840					1:18.8	
	1921	Sept. 20	20.32	.0	20.32	.622	1.162	.259	.903		1:31.4	35 per cent of a crop.
	1922	Sept. 19	15.16	.20	15.36	.678	1.295	.648	.647		1:22.6	
	1923	Sept. 21	18.94	.0	18.94	.687	.897	.367	.530	20.16	1:27.1	
Canada	1919	Sept. 22	17.42	.0	17.42	.952				18.98	1:18.3	
	1920	Oct. 15	18.32	.0	18.32	1.125					1:16.2	
	1921	Sept. 24	14.93	.07	15.00	.975	1.564	.406	1.158		1:15.3	30 per cent of a crop.
	1922	Sept. 28	13.86	.0	13.86	1.150	1.389	.733	.656		1:11.2	
	1923	Oct. 2	16.49	.87	17.36	.993	.820	.196	.624	18.98	1:17.4	
Catawba	1919	Oct. 7	20.66	.0	20.66	.945					1:21.8	
	1920	Oct. 16	16.49	1.91	18.40	.820				21.64	1:22.4	
	1921	Sept. 28	16.85	.03	16.88	1.087	1.539	.548	.981		1:15.6	75 per cent of a crop.
	1922	Sept. 27	15.60	1.04	16.64	1.093	1.156	.258	.898		1:15.3	Did not color well.
	1923	Oct. 6	18.37	.65	19.02	1.233	1.316	.547	.769	19.94	1:15.4	
Clevener	1919	Oct. 3	13.74	.0	13.74	1.222				16.21	1:10.6	
	1920	Oct. 12	15.46	.0	15.46	1.425					1:10.8	
	1921	Sept. 28	11.91	.78	12.69	1.335	2.462	.559	1.903		1:9.5	About 75 per cent of a crop.
	1922	Sept. 26	12.72	.69	13.41	1.766	1.821	.432	1.389		1:7.6	
	1923	Oct. 6	13.00	.10	13.10	2.097	2.671	.987	1.684	15.48	1:6.2	

Clinton	1919	Oct. 7	22.44	.0	22.44	1.530					1:14.0	
	1920	Oct. 9	19.96	.0	19.96	1.687				21.46	1:11.7	
	1921	Sept. 27	16.40	.62	17.02	1.665	1.720	.410	1.310		1:10.2	About 65 per cent of a crop.
	1922	Sept. 21	17.03	1.22	18.25	1.760	1.752	.544	1.208		1:10.4	
	1923	Oct. 10	19.62	1.30	20.92	1.653	1.111	.076	1.035	21.78	1:13.2	
Colerain	1919	Sept. 18	12.78	2.84	15.62	.675					1:23.1	
	1920	Sept. 22	12.76	.0	12.76	.937				16.21	1:13.6	
	1921	Sept. 19	16.02	.32	16.34	.750	1.267	.394	.873		1:21.7	35 per cent of a crop.
	1922	Sept. 14	12.54	.0	12.54	.792	1.234	.415	.819		1:15.5	
	1923	Sept. 25	15.18	1.80	16.98	.648	1.051	.479	.572	17.99	1:26.2	
Concord	1919	do	15.02	.0	15.02	.795				16.97	1:16.3	
	1920	do	13.23	.21	13.44	.825					1:16.1	
	1921	Sept. 16	14.13	.16	14.29	.915	1.273	.397	.876		1:15.6	65 per cent of a crop.
	1922	Sept. 21	13.91	.08	13.99	1.178	1.873	.872	1.001		1:10.9	
	1923	Sept. 19	14.68	.52	15.20	.767	.936	.440	.496	16.52	1:19.8	
Cynthiana	1919	Oct. 6	17.46	.0	17.46	1.285					1:13.6	
	1920	Oct. 7	16.50	.0	16.50	1.335				18.04	1:12.4	
	1921	Sept. 27	14.46	4.65	19.11	1.155	1.276	.253	1.023		1:16.4	Normal crop.
	1922	Sept. 26	17.02	.75	17.77	1.732	1.398	.499	.899		1:10.2	
	1923	Oct. 8	16.90	1.92	18.82	1.269	1.222	.538	.684	19.86	1:14.8	
Dakota	1919	Oct. 5	12.22	.08	12.30	.787				15.07	1:15.6	
	1920											Normal crop but no samples secured.
	1921	Sept. 24	13.74	.0	13.74	1.365	1.372	.211	1.161		1:10.1	50 per cent of a crop.
	1922	Sept. 16	14.86	.10	14.96	1.463	1.450	.423	1.027		1:10.0	
	1923	Sept. 22	15.14	1.65	17.39	1.299	.880	.171	.709	19.08	1:13.4	
Diamond	1919	do	17.16	.0	17.16	.990					1:17.1	
	1920	Sept. 18	13.74	.0	13.74	.885				16.06	1:15.5	
	1921	Sept. 19	14.76	.0	14.76	.787	1.263	.394	.869		1:18.2	20 per cent of a crop.
	1922	Sept. 16	11.63	.29	11.92	.908	1.571	.648	.923		1:12.9	
	1923	Sept. 17	12.70	.55	13.25	1.109	.940	.174	.766	13.98	1:11.9	
Diana	1919	Sept. 23	17.28	.0	17.28	.690				19.00	1:24.7	
	1920	Oct. 13	16.94	.0	16.94	1.012					1:16.7	Did not color normally.
	1921	Sept. 29	18.25	.0	18.25	.795	.993	.303	.690		1:22.9	25 per cent of a crop.
	1922	Sept. 27	16.28	.68	16.96	.968	.899	.174	.725		1:17.5	
	1923	Oct. 10	15.72	.0	15.72	.897	.581	.205	.376	16.74	1:17.0	Did not color well.
Diogenes	1919	Sept. 24	14.51	.75	15.26	1.485					1:10.2	
	1920	Oct. 6	15.74	.0	15.74	.982				18.21	1:16.0	
	1921	Sept. 28	14.03	.42	14.45	1.230	2.023	.594	1.429		1:11.7	65 per cent of a crop.
	1922	Sept. 25	14.29	.53	14.82	1.803	1.303	.336	.967		1: 8.2	
	1923	Oct. 7	15.69	1.35	17.04	1.743	1.812	.427	1.385	18.41	1: 9.8	
Delaware	1919	Sept. 18	16.82	.39	17.21	.832				21.21	1:20.7	
	1920	Sept. 23	17.25	.0	17.27	.840					1:20.5	
	1921											Partial crop picked prematurely and no samples secured.
	1922	Sept. 20	18.36	.0	18.36	.696	.730	.176	.554		1:25.6	
	1923	Sept. 26	24.80	.45	25.25	.770	.684	.154	.530	26.08	1:32.8	Overripe.
Dutchess	1919	Oct. 1	17.03	.0	17.00	.526				18.93	1:32.3	
	1920	Oct. 14	18.72	.0	18.72	.731					1:25.6	
	1921	Sept. 26	15.76	.0	15.76	.735	.873	.222	.651		1:20.2	
	1922	do	14.68	.14	14.82	.900	1.260	.561	.699		1:16.4	
	1923	Oct. 1	15.76	1.77	17.53	.527	.538	.136	.402	19.58	1:29.8	

[a] The total sugar after inversion is occasionally slightly less than the free reducing sugar, due to a small loss of levulose during inversion.

TABLE III.—*Analytical data for 49 varieties fruiting annually, 1919 to 1923, at Vineland, N. J.*—Continued

	Year	Date picked	Reducing sugar	Cane sugar	Total sugar after inversion	Acid as tartaric	Total astringency	Tannin	Non-tannin	Total solids	Acid-sugar ratio	Remarks
							Grams per liter	*Grams per liter*	*Grams per liter*			
Elvira	1919	Sept. 19	14.84	.0	14.84	1.290				16.64	1:11.5	Does not ripen uniformly; cracks and rots.
	1920	Sept. 29	14.54	.0	14.54	.847					1:17.1	
	1921	Sept. 17	14.26	.43	14.69	.998	.944	.231	.713		1:12.3	50 per cent of a crop.
	1922	Sept. 16	13.54	.0	13.54	1.011	1.484	.854	.630		1:12.8	
	1923	Sept. 24	15.38	.0	15.38	.775	.662	.175	.487	16.32	1:19.2	
Eumelan	1919	Sept. 29	22.04	1.09	23.13	.555					1:41.7	
	1920	Oct. 4	19.61	.0	19.61	.607				21.64	1:32.3	
	1921	Sept. 29	16.02	.0	16.02	.660	.994	.168	.826		1:23.7	75 per cent of a crop.
	1922	Sept. 25	18.28	1.12	19.40	.761	1.346	.250	1.096	21.93	1:25.5	
	1923	Oct. 7	17.84	2.18	20.02	.828	.768	.178	.590		1:24.1	
Franklin	1919	Oct. 3	15.06	.0	15.06	1.200					1:11.7	
	1920	Oct. 11	13.10	.36	13.46	1.380				16.83	1: 9.7	
	1921	Sept. 28	8.80	3.03	11.83	1.415	1.693	.196	1.497		1: 8.3	Unaffected by frost.
	1922	Sept. 26	11.96	.0	11.96	1.593	1.605	.388	1.217		1: 7.5	
	1923	Oct. 6	16.78	.92	17.70	1.689	1.684	.359	1.325	18.92	1:10.5	
Goethe	1919	Oct. 1	13.75	.05	13.80	.945				16.21	1:14.6	
	1920	Oct. 4	12.25	2.04	14.29	.862					1:16.5	
	1921	Sept. 22	16.00	.0	16.00	.765	1.002	.404	.598		1:20.9	40 per cent of a crop.
	1922	Sept. 23	14.98	.0	14.98	.880	1.450	.751	.699		1:16.7	
	1923	Sept. 27	14.46	.13	14.59	.803	.984	.471	.513	16.08	1:18.1	
Herbemont	1919	Oct. 6	15.02	.18	15.20	1.241				17.71	1:12.2	
	1920	Oct. 16	16.64	.0	16.64	1.425					1:11.7	
	1921	Sept. 28	15.97	.26	16.23	1.192	1.221	.266	.955		1:13.6	
	1922	Oct. 2	16.70	.06	16.76	1.272	1.303	.604	.699		1:13.1	
	1923	Oct. 7	15.38	1.85	17.23	1.311	.761	.368	.393	18.56	1:13.1	
Herbert	1919	Sept. 29	13.62	1.21	14.83	.735				16.29	1:20.2	
	1920	Sept. 27	15.66	.0	15.66	1.072					1:14.6	
	1921	Sept. 22	14.20	.88	15.08	.840	1.293	.417	.876		1:17.9	65 per cent of a crop.
	1922	Sept. 23	16.02	.0	16.02	.982	1.173	.258	.915		1:16.1	
	1923	Sept. 24	17.39	.0	17.39	.822	.940	.248	.692	18.86	1:21.1	
Isabella	1919	Sept. 29	13.85	2.79	16.64	.780				18.23	1:21.3	
	1920	Oct. 15	12.25	4.10	16.35	1.087					1:15.0	
	1921	Sept. 29	17.56	.0	17.56	.900	.926	.322	.604		1:19.5	
	1922	Sept. 28	16.18	.44	16.62	1.195	.949	.242	.707		1:14.0	
	1923	Oct. 6	16.90	1.63	18.53	1.327	.932	.359	.573	18.84	1:13.9	
Ives	1919	Sept. 25	15.67		15.67	.547					1:28.0	Overripe and shrivelled.
	1920	Sept. 18	13.85	.04	13.89	.750					1:18.5	
	1921											
	1922	Sept. 19	11.74	1.22	12.96	1.424	1.856	.605	1.251	16.24	1: 9.1	
	1923	Sept. 16	13.59	2.80	16.39	.772	1.008	.136	.872	17.58	1:20.7	

Jefferson	1919	Sept. 23	17.50	-------	17.50	.787	----------	----------	----------	19.62	1:22.2	
	1920	Oct. 4	12.25	2.01	14.26	.765	----------	----------	----------	----------	1:18.6	
	1921	Sept. 24	13.11	-------	13.11	.652	.933	.309	.622	----------	1:20.0	60 per cent of a crop.
	1922	Sept. 18	11.57	.80	12.37	.752	1.199	.250	.949	----------	1:16.4	Cracking and rotting badly, hence picked slightly underripe.
	1923	Oct. 8	17.96	2.45	20.41	.835	.761	.163	.598	21.92	1:24.4	
Lampasas	1919	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	Crop picked prematurely; no samples secured.
	1920	Sept. 25	14.79	0.0	14.79	.750	----------	----------	----------	17.21	1:19.6	
	1921	Sept. 19	15.49	2.05	17.54	.742	.861	.057	.804	----------	1:23.6	30 per cent of a crop.
	1922	Sept. 21	13.74	.40	14.14	.701	1.148	.130	1.018	----------	1:20.1	
	1923	Oct. 6	12.78	1.70	14.48	.944	.863	.242	.621	15.72	1:15.3	
Lenoir	1919	---do----	11.98	2.71	14.69	1.650	----------	----------	----------	18.89	1: 8.9	
	1920	Oct. 15	14.43	1.70	16.13	1.560	----------	----------	----------	----------	1:10.3	
	1921	Sept. 28	16.64	-------	16.64	1.560	1.523	.417	1.106	----------	1:10.6	Normal crop.
	1922	Oct. 2	13.72	0.0	13.72	2.047	1.027	.388	.639	----------	1: 6.6	
	1923	Oct. 6	15.84	1.74	17.58	1.537	.970	.432	.538	19.52	1:11.4	
Lindley	1919	Sept. 29	20.36	0.0	20.36	.547	----------	----------	----------	21.89	1:37.1	
	1920	Sept. 27	14.61	0.0	14.61	.675	----------	----------	----------	----------	1:21.6	
	1921	Sept. 22	14.02	.98	15.00	.605	1.326	.167	1.159	----------	1:24.8	60 per cent of a crop.
	1922	---do----	15.12	.40	15.52	.735	1.778	.346	1.432	----------	1:21.0	
	1923	Sept. 26	17.30	.22	17.52	.739	.880	.256	.624	18.32	1:23.7	
Lucile	1919	Sept. 20	18.35	0.0	18.35	.630	----------	----------	----------	----------	1:29.1	
	1920	---do----	14.06	0.0	14.06	.750	----------	----------	----------	----------	1:18.7	
	1921	Sept. 19	13.14	1.68	14.82	.600	1.441	.279	1.162	18.61	1:24.7	50 per cent of a crop.
	1922	Sept. 14	11.66	.67	12.33	.684	1.312	.363	.949	----------	1:18.4	
	1923	Sept. 18	14.60	.76	15.36	.764	.957	.316	.641	16.48	1:20.1	
Martha	1919	Sept. 20	17.37	.13	17.50	.570	----------	----------	----------	----------	1:30.7	
	1920	---do----	19.02	0.0	18.91	.600	----------	----------	----------	20.20	1:31.6	
	1921	Sept. 17	17.97	1.04	19.01	.547	1.299	.313	.986	----------	1:34.7	25 per cent of a crop.
	1922	Sept. 15	16.66	.31	16.97	.537	1.449	.413	1.036	----------	1:31.6	
	1923	---do----	15.83	.23	16.06	.621	1.226	.264	.962	19.04	1:25.8	
Massasoit	1919	Sept. 29	14.67	0.0	14.67	.570	----------	----------	----------	----------	1:25.7	
	1920	Sept. 24	12.49	0.0	12.46	.990	----------	----------	----------	----------	1:12.5	
	1921	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	
	1922	Sept. 22	13.02	.42	13.44	.908	1.994	.648	1.346	15.02	1:14.9	
	1923	Oct. 1	14.26	0.0	14.21	.629	.812	.352	.470	15.00	1:22.6	
Merrimac	1919	Sept. 22	13.74	3.08	16.82	.720	----------	----------	----------	----------	1:23.3	
	1920	---do----	15.84	0.0	15.84	.825	----------	----------	----------	17.83	1:19.2	
	1921	Sept. 24	17.67	0.0	17.54	.829	1.064	.281	.783	----------	1:21.0	Do.
	1922	Sept. 21	13.38	.16	13.54	.886	1.148	.329	.819	----------	1:14.9	
	1923	Sept. 23	17.29	.02	17.31	.855	.829	.231	.598	18.80	1:20.2	
Missouri Riesling	1919	Sept. 20	18.66	0.0	18.66	1.080	----------	----------	----------	----------	1:17.0	
	1920	Sept. 29	17.70	.62	17.72	1.125	----------	----------	----------	19.23	1:15.7	
	1921	Sept. 19	17.92	0.0	17.92	1.050	.762	.161	.601	----------	1:17.0	Unaffected by frost.
	1922	Sept. 18	13.64	0.0	13.61	.989	1.286	.855	.431	----------	1:13.7	
	1923	Sept. 20	15.92	.20	16.12	.891	.581	.060	.521	17.30	1:17.8	
Montefiore	1919	Sept. 24	19.00	0.0	18.93	.750	----------	----------	----------	----------	1:25.1	
	1920	---do----	16.11	0.0	16.01	.637	----------	----------	----------	18.21	1:25.1	
	1921	Sept. 20	15.97	1.53	17.50	.780	1.718	.554	1.164	----------	1:22.4	60 per cent of a crop.
	1922	---do----	15.28	.30	15.58	1.082	2.511	1.001	1.510	----------	1:14.4	
	1923	Sept. 25	16.79	.92	17.71	.555	1.128	.410	.718	19.88	1:31.9	

TABLE III.—*Analytical data for 49 varieties fruiting annually, 1919 to 1923, at Vineland, N. J.*—Continued

	Year	Date picked	Reducing sugar	Cane sugar	Total sugar after inversion	Acid as tartaric	Total astringency	Tannin	Non-tannin	Total solids	Acid-sugar ratio	Remarks
							Grams per liter	*Grams per liter*	*Grams per liter*			
Nectar	1919	Sept. 23	17.78	0.0	17.78	.757					1:23.3	
	1920	Sept. 20	17.60	0.0	17.52	1.072					1:16.2	
	1921	Sept. 24	13.04	2.93	15.97	.982	1.910	.629	1.281	19.04	1:16.2	Normal crop.
	1922	Sept. 22	13.94	.15	14.09	1.289	4.012	2.347	1.665		1:10.9	Immature.
	1923	Sept. 16	18.58	1.59	20.17	.833	1.410	.709	.701	21.54	1:24.2	Overripe.
Niagara	1919	Sept. 25	15.54	.24	15.78	.622					1:25.3	
	1920	Sept. 29	15.98	0.0	16.00	.705				18.83	1:22.7	
	1921	Sept. 20	17.00	0.0	17.00	.607	1.267	.141	1.126		1:28.0	
	1922	Sept. 21	14.25	.33	14.58	.664	1.182	.189	.993		1:21.9	
	1923	Sept. 22	15.54	.56	16.10	.750	.778	.129	.649	17.50	1:21.6	
Noah	1919	Sept. 25	15.12	1.02	16.14	1.462					1:11.0	
	1920	Sept. 30	17.26	1.47	18.73	1.127				20.12	1:16.6	
	1921	Sept. 23	16.87	0.0	16.87	1.230	1.004	.111	.893		1:13.7	Normal crop.
	1922	Sept. 22	17.09	.53	17.62	1,178	1.320	.327	.993		1 14.9	
	1923	Oct. 1	18.64	.91	19.55	1.212	.718	.282	.436	20.76	1:16.1	
Norton	1919	Oct. 6	21.47	.0	21.47	1.567					1:13.7	
	1920	Oct. 8	19.17	.0	19.16	1.462				21.40	1:13.1	
	1921	Sept. 27	20.80	.0	20.80	1.200	1.443	.381	1.062		1:17.3	Do.
	1922	Sept. 28	18.28	1.10	19.38	1.828	1.562	.475	1.087		1:10.6	
	1923	Oct. 3	17.70	2.54	20.54	1.148	1.248	.453	.795	21.96	1:17.6	
Perkins	1919	Sept. 22	13.48	.0	13.48	.600				15.04	1:22.5	
	1920	Sept. 18	12.42	.0	12.40	.840					1:14.8	
	1921	Sept. 27	9.82	3.39	13.21	.690	1.269	.165	1.104		1:19.1	50 per cent of a crop.
	1922	Sept. 18	9.50	1.51	11.01	.673	1.286	.423	.863		1:16.3	
	1923	Oct. 1	13.73	.70	14.43	.555	.897	.282	.615	15.20	1:26.0	
Pocklington	1919	Sept. 26	15.79	.0	15.63	.660					1:23.6	
	1920	Oct. 2	13.25	1.61	14.86	.787					1:18.9	
	1921	Sept. 20	16.10	.50	16.60	.720	1.321	.220	1.101	18.31	1:23.0	
	1922	Sept. 15	11.98	.87	12.85	1.067	1.554	.605	.949		1:12.0	
	1923	Sept. 25	13.17	1.76	14.93	.751	.983	.496	.487	15.82	1:19.8	
Rommel	1919	Sept. 22	11.72	1.57	13.29	.822					1:16.1	
	1920	Sept. 28	10.98	2.54	13.52	.840					1:16.1	
	1921	Sept. 26	15.41	.0	15.41	.817	.944	.210	.734	17.26	1:18.8	35 per cent of a crop.
	1922	Sept. 19	13.18	.46	13.64	.977	1.199	.232	.967		1:13.9	
	1923	Oct. 3	12.76	1.80	14.56	.659	.769	.333	.436	16.30	1:22.0	
Salem	1919	Sept. 26	21.58	.0	21.58	.780				22.98	1:27.6	
	1920	Sept. 23	13.50	2.70	16.20	1.050					1:15.4	
	1921	Sept. 21	15.94	.22	16.16	.862	1.234	.288	.946		1:18.7	75 per cent of a crop.
	1922	Sept. 23	16.90	.72	17.62	.923	1.665	.811	.854	18.98	1:19.1	
	1923	Oct. 2	17.91	.94	18.85	.770	.786	.303	.483	19.78	1:24.4	

Ulster	1919	Sept. 22	16.67	.0	16.67	.822				19.82	1:20.3	
	1920	Oct. 1	12.26	3.18	15.44	.975					1:15.8	
	1921	Sept. 21	14.54	1.68	16.22	.813	.804	.041	.763		1:20.0	
	1922	---do---	16.90	.68	17.58	.965	1.182	.362	.820		1:18.2	
	1923	Oct. 4	19.90	.07	19.97	.748	.709	.060	.649	21.04	1:26.7	
Vergennes	1919	Sept. 30	18.84	.0	18.84	.822				20.21	1:22.9	
	1920	Oct. 9	14.62	.26	14.88	.870					1:17.1	
	1921	Sept. 21	19.26	.0	19.20	1.042	1.020	.209	.811		1:18.4	Normal crop.
	1922	Sept. 23	15.08	.0	15.08	.795	1.009	.396	.613		1:18.7	
	1923	Oct. 5	18.70	.0	18.64	.715	.709	.213	.496	19.66	1:25.5	
Wilder	1919	Sept. 30	20.28	.0	20.28	.570					1:35.1	
	1920	Sept. 23	12.10	2.48	14.58	1.267				17.38	1:11.5	
	1921	Sept. 21	12.73	1.95	14.68	.765	1.001	.141	.860		1:19.2	Do.
	1922	Sept. 23	14.38	.0	14.38	.841	1.122	.294	.828		1:16.7	
	1923	Oct. 5	15.84	.61	16.45	.753	1.154	.214	.940	18.64	1:21.8	
Woodruff	1919	Sept. 26	14.53	.0	14.48	.630				16.54	1:22.9	
	1920	Oct. 2	14.26	.0	14.20	.787					1:18.0	
	1921	Sept. 20	15.24	.43	15.67	.757	1.228	.195	1.033		1:20.7	
	1922	Sept. 16	11.79	2.62	14.41	.803	1.407	.354	1.053		1:17.9	
	1923	Sept. 22	14.74	.83	15.57	.646	.743	.247	.496	16.58	1:24.1	
Worden	1919	Sept. 19	9.75	4.67	14.32	.967					1:14.8	
	1920	Sept. 25	10.03	5.57	15.60	.975					1:16.0	
	1921	Sept. 16	14.79	.12	14.91	.582	1.369	.357	1.012	16.34	1:25.6	45 per cent of a crop; over ripe.
	1922	Sept. 15	11.44	.0	11.41	1.070	1.665	.612	1.053		1:10.7	
	1923	Sept. 20	11.34	1.46	12.80	1.062	.949	.222	.727	13.94	1:12.0	

TABLE IV.—*Analytical data for varieties not fruiting regularly during 1918–1923*

	Year	Date picked	Reducing sugar	Cane sugar	Total sugar	Acid as tartaric	Total astringency	Tannin	Non-tannin	Total solids	Acid-sugar ratio	Remarks
							Grams per liter	*Grams per liter*	*Grams per liter*			
Campbell	1919	Sept. 26	16.43	0.0	16.43	0.592					1:27.7	Very small crop; vines in poor condition.
	1920	Oct. 1	9.92	3.73	13.65	.675					1:20.2	
	1921	Sept. 20	14.34	.0	14.18	.645	0.813	0.087	0.726	16.21	1:22.2	About 40 per cent of a crop.
	1922	Sept. 15	12.78	.53	13.31	.679	1.122	.216	.906		1:19.5	
	1923	Sept. 17	14.32	1.65	15.97	.679	.880	.333	.547		1:23.5	
Centennial	1919	Sept. 22	12.68	.86	13.54	.975					1:13.9	
	1920											No crop.
	1921	Sept. 24	14.71	.0	14.71	1.185	1.840	.619	1.221		1:12.4	30 per cent of a crop.
	1922	Sept. 14	11.36	.08	11.44	1.207	1.449	.535	.914	14.64	1: 9.5	
	1923	Oct. 2	17.15	2.05	19.20	1.382	.914	.266	.648		1:13.9	Overripe and shriveled.
Champion	1921	Sept. 19	12.40	.0	12.24	1.215	1.193	.147	1.046	13.06	1:10.0	
	1922	Sept. 14	8.96	.41	9.37	.667	2.046	.976	1.070		1:14.0	Badly overripe.
	1923	Sept. 23	11.92	.05	11.97	1.294	1.043	.385	.658	12.92	1: 9.3	
"Dutchess seedling"	1922	Sept. 28	14.34	.02	14.36	.669	.220	.044	.176		1:21.4	No crop prior to 1922.
	1923	Oct. 2	11.98	1.87	13.85	.656	.547	.086	.461	15.82	1:20.1	
Early Daisy	1919	Sept. 23	12.14	.9	12.23	.480				14.44	1:25.4	Not well matured.
	1920											No crop.
	1921											Do.
	1922	Sept. 18	15.97	.0	15.96	.812	3.280	1.117	2.114		1:19.6	
	1923	Sept. 23	18.34	2.50	20.84	.673	1.442	.685	.757	21.30	1:30.9	Very over ripe and soft; juice viscuous.
Early Victor	1919	do	14.36	.05	14.41	.525					1:27.4	
	1920	Sept. 20	15.28	.0	15.28	.753					1:20.3	
	1921											No crop.
	1922	Sept. 14	13.49	.0	13.41	.756	1.053	.234	.819	19.08	1:17.7	
	1923	Sept. 20	13.06	1.79	14.85	1.037	.936	.557	.479	16.93	1:14.3	
Eaton	1919	Sept. 23	10.49	.87	11.36	.877				14.20	1:12.9	Never bears more than a few scattered clusters.
	1920											No crop.
	1921											Do.
	1922	Sept. 14	11.94	.0	11.94	1.160	.704	.378	.326	14.64	1:10.3	
	1923	Sept. 20	13.06	1.79	14.85	1.037	.936	.457	.479		1:14.3	
Gaertner	1919											Do.
	1920	Sept. 27	14.12	.0	14.06	.795					1:17.7	Never bears more than occasional clusters.
	1921											No crop.
	1922	Sept. 22	13.42	.0	13.32	.843	1.752	.535	1.217		1:15.8	Colors very poorly.
	1923	Oct. 1	15.98	.0	15.95	.773	.607	.086	.521	17.66	1:24.5	
Hartford	1922	Sept. 14	12.42	.24	12.66	.695	1.596	.682	.914		1:18.2	No crop prior to 1922.
	1923	Sept. 19	13.32	2.74	16.06	.654	1.043	.342	.701		1:24.5	

King Philip	1920	Oct. 1	10.70	3.26	13.96	.750					1:18.6	Scattered bunches; poorly ripened
	1921	Sept. 21	14.76	.0	14.71	.743	.778	.052	.726		1:19.8	
	1922	Sept. 20	14.00	.0	14.00	.826	1.027	.034	.993		1:16.9	
	1923	Sept. 26	17.56	.0	17.42	.817	.769	.316	.453	18.08	1:21.3	
Lady	1919	Sept. 23	14.79	.23	15.02	.682					1:22.0	Fails to mature evenly.
	1920	Sept. 18	13.29	2.57	15.86	.885					1:17.9	
	1921	Sept. 19	17.96	.0	17.91	.787	.951	.038	.813	19.01	1:22.7	Scattered bunches only.
	1922											No crop.
	1923											Do.
Moore	1919	Sept. 18	11.52	1.34	12.86	.915					1:14.0	
	1920											Do.
	1921											Do.
	1922											Do.
	1923	Sept. 20	12.82	1.08	13.90	.991	1.301	.385	.916	14.96	1:14.0	
Rebecca	1919											Do.
	1920											Do.
	1921											Do.
	1922	Sept. 21	13.39	.70	14.09	.650	1.208	.259	.949		1:21.7	
	1923	Oct. 10	12.61	1.13	13.74	.519	.675	.205	.470	14.64	1:26.4	
"Seibel Hybrid No. 1"	1920	Oct. 13	19.14	.0	19.10	1.440					1:13.2	
	1921	Sept. 27	14.67	.0	14.67	1.417	1.258	.304	.954		1:10.3	
	1922	Sept. 28	13.36	1.22	14.58	1.550	1.631	.612	1.019	17.23	1: 9.4	
	1923	Oct. 2	16.36	.0	16.36	1.587	1.282	.342	.940	17.86	1:10.2	
"Seibel Hybrid No. 2"	1920	Oct. 13	13.38	.0	13.38	1.657				16.52	1: 8.0	
	1921	Sept. 27	12.20	1.52	13.72	1.305	1.423	.403	1.020		1:10.5	
	1922	Sept. 29	14.38	.33	14.71	1.871	1.795	.923	.872		1: 7.8	
	1923	Oct. 8	14.88	1.30	16.18	1.821	1.436	.419	1.017	17.06	1: 8.8	
Winchell	1919	Sept. 21	22.60	.0	22.60	.465				23.13	1:48.6	Overripe and shriveled.
	1920											
	1921											
	1922											
	1923	Sept. 18	20.20	.66	20.86	.496	.889	.325	.564	21.88	1:42.0	Overripe.

American grapes grown in Ohio, New York, Virginia, Michigan, Pennsylvania, and New Jersey in the years 1908–1913. The number of samples of a variety analyzed by these workers varied from 1 to 15 for the less important varieties to 264 for Catawba, 50 for Clinton, 303 for Concord, 95 for Delaware, 125 for Ives, and 116 for Niagara. These were collected over a period of several years, the samples in some cases containing collections made in the same locality for two or more years in succession and in the case of the more widely grown varieties may be considered to fairly represent their composition as grown in the commercial grape-growing districts of the Central and Eastern States. Naturally no data as to the conditions of the various vineyards represented, or the climatic conditions during the period of the work could be obtained in an experiment covering so large an area, but it is noted (*3*) that in the Ohio grape district the year 1908 was an exceptionally favorable one, that 1909 was characterized both by a very heavy crop and by unpropitious weather during the ripening period, and that 1910 was a year of partial crop failure as a result of late spring frosts. Hence the samples represent a wide range of seasonal conditions and of load on the vines as well as of soils and cultural practices and may be expected to show the extremes encountered in the area represented in the work. The samples were in part taken at wine cellars as the fruit was delivered by wagon or rail, and in part shipped to the laboratory after having been collected by the writers, by cooperating members of Agricultural Experiment Station forces, or by growers, and some errors of sampling were inevitable. The extreme range in sugar content found by Alwood and his associates in the six leading varieties, as assembled by the writer from their published data, were as follows: Catawba—from 12.92 to 22.53 per cent, or 9.61 per cent difference; Clinton—from 11.52 to 23.24 per cent, a range of 11.72 per cent difference; Concord—from 10.41 to 20.48 per cent, a range of 10.07 per cent difference; Delaware—from 16.20 to 26.85 per cent, a range of 10.65 per cent difference; Ives—from 11.17 to 21.22 per cent, a range of 10.05 per cent difference; and Niagara—11.36 to 19.24 per cent, a range of 7.88 per cent difference. While the maximum and minimum figures found for a variety usually occur in different years and in more or less widely separated districts, in the case of Concord they occurred in a group of 23 samples collected at North East, Pa., in 1910. With this exception, the samples of a variety for any given locality and year show much narrower differences, as would be expected.

To sum up by a generalization, the range of differences in percentage of sugar content found by Alwood and his associates in samples of grapes collected from a large number of sources in six States over a five-year period is approximately twice as great as that found by the author in the fruit from one vineyard over a like period. The absolute amounts of sugar present as reported by Alwood are uniformly higher than those found by the writer for the same varieties. This difference is due in part, undoubtedly, to the difference in the method of sampling, Alwood's samples having been crushed and pressed through cheesecloth by hand. It may indicate, however, that grapes grown in the Vineland district of New Jersey regularly develop a somewhat lower sugar content than in the Lake Erie and Chautauqua districts. Alwood and his associates (*7*) analyzed a number of samples from New Jersey in 1913, among them 24 varieties from the Vineland vineyard, and they comment upon them as being low in sugar as compared with the same varieties grown elsewhere. It would require a very comprehensive study, employing rigidly controlled methods of sampling, to definitely determine whether there is a constant difference in composition of a given variety in different localities throughout its range.

PRESENCE OF CANE SUGAR

The literature dealing with the chemistry of the European grape, *Vitis vinifera*, is in agreement that sucrose is never found in the mature fruit of this species. Such standard works on wine making as Babo and Mach (*11*), Thudichum (*57*), and Laborde (*40*), repeatedly make this statement, and the detailed studies of Famintzin (*21*), Girard and Lindet (*23*), Martinand (*46*), Baragiola and Godet (*12*), and Roos and Hugues (*54*), confirm it. Martinand found invertase present in all parts of vine and fruit and could find no saccharose either in free-run juice or in the pulp of the ripe fruit. Girard and Lindet and Baragiola and Godet found traces of saccharose in developing grapes, none in mature fruit. Roos and Hugues studied the dextrose-levulose ratio in various Vinifera varieties and also in the American varieties Con-

cord, Clinton, Elvira, Isabella, Jacques (Lenoir) Noah, Taylor, Telegraph, and Othello, grown at the oenological station at Montpelier, finding no sucrose present in any of them. Bioletti, Cruess, and Davi (*14*) also found no sucrose in Vinifera varieties grown in California.

Most American students of grapes have proceeded upon the assumption that the native American varieties are like the European Vinifera in being sucrose free, and in the older analytical work and some of more recent date (*13, 26, 42, 53, 61*) upon American varieties no examinations for sucrose were made. It has even been proposed to employ the absence of sucrose as a test for purity in unfermented grape juices (*58*).

The presence of sucrose in considerable amounts in well matured samples of fruit has, however, been reported for a number of American grapes. Gore (*24*) in 1909 found 2 per cent in juice of Mish and 0.69 per cent in Scuppernong juice (two varieties of *Vitis rotundifolia* or "Muscadine" grapes), with probable traces in Concord and Catawba juices, and Alwood (*1, 2, 4, 8*) in the next two years found large amounts in an unnamed seedling with smaller amounts in Catawba, Norton, and Montefiore, Hayes, Worden, Pocklington, Illinois City, and Nectar. Its presence was not correlated with degree of maturity of the fruit. Howard (*34*) reported analyses of 12 samples of grape juice, finding cane sugar in Catawba and in three unlabeled samples which were presumably Concord. Thompson and Whittier (*56*) found 0.5 per cent or more of sucrose in the juice of 5 varieties of grapes examined. Gore (*25*) examined 66 varieties yearly for four years (1911–1914), finding that it was present occasionally in 10, frequently in 13, and absent in 43. The fruit examined by Gore came from the Vineland vineyard, but a number of varieties of the Muscadine grape (*Vitis rotundifolia*) grown at Willard, N. C., were also examined. No sucrose was found at any time in Agawam, Barry, Berckmans, Brighton, Brilliant, Canada, Catawba, Centennial, Clinton, Concord, Cynthiana, Delaware, Diogenes, Dutchess, Early Champion, Eaton, Elvira, Eumelan, Franklin, Gaertner, Goethe, Hartford, Herbemont, "Seibel Hybrid No. 1 or No. 2," Iona, Ives, Jefferson, Lampasas, Lindley, Martha, Massasoit, Merrimac, Missouri Riesling, Montefiore, Niagara, Noah, Norton, Perkins, Salem, Ulster or the Rotundifolia varieties Flowers and James. It was present in all four years in Thomas (Rotundifolia) Pocklington and Nectar; in three years in Early Victor, Lady, Moore Early, Scuppernong (Rotundifolia) and Woodruff; two years in Campbell Early, Colerain, Eden (Rotundifolia), Herbert, and Lenoir; and one year only in Clevener, Diamond, Diana, Early Daisy, Isabella, Rommel, Vergennes, Wilder, Winchell, and Worden. Some of the grapes used were pressed cold and some were heated to 95° C. before pressing, after shipment from the vineyard to Washington. The widespread but very irregular occurrence of sucrose in American grapes is therefore firmly established.

In the course of the present work indications of the presence of at least a trace of cane sugar have been found at some time in every one of the 66 varieties under study except Gaertner. In a few cases the difference in the figures before and after inversion is so slight—0.20 per cent or less—as to make it probable that the differences are due to experimental error. This may be the case for the small amounts found in Brilliant and Champion in 1922, Delaware in 1923, Elvira in 1921, Massasoit in 1922, Missouri Riesling and Vergennes in 1920, as in all these varieties indications of cane sugar appeared only once in the period, but in all these cases the repetition of the test confirmed the first figures obtained. Of the 66 varieties fruiting in 1923, 48 showed positive amounts (0.20 per cent or more) of sucrose; in 1922, 37 out of 63 showed positive amounts; in 1921, 23 out of 53; in 1920, 21 out of 66; and in 1909, 20 out of 59. As the determinations for 1919, 1920, and 1921 were made upon previously pasteurized samples, there is a possibility for the inversion of small amounts of cane sugar where present during the pasteurization. The fact that many of the juices of the varieties from the same vineyard previously examined by Gore had been prepared by heating the fruit prior to pressing may have been responsible for the negative results reported by him for many of these varieties.

That the occurrence of sucrose is exceedingly erratic is indicated by Gore's results as well as by those here reported. In no variety was it invariably present through the five-year period covered by the work. In 15 varieties it occurred only once—Brighton, Brilliant, Canada, Champion, Diana, Delaware, Dutchess, Early Daisy, Early Victor, King Philip, Massasoit, Merrimac, Missouri Riesling, Vergennes, Winchell—and in 7 of these only in 1923. In 16 varieties it was found twice, in 21 varieties 3 times. It was present in four years out of five in the 11 varieties—Ca-

tawba, Diogenes, Franklin, Isabella, Lucile, Noah, Pocklington, Rommel, Salem, Wilder, and Worden. The analyses for three of the five years were made upon previously pasteurized juices and the negative results for cane sugar were obtained with pasteurized samples in which there is a possibility of inversion of cane sugar during pasteurization. This indicates strongly that cane sugar may be normally present in the fresh juices of these varieties. Franklin may be an exception, as the fresh juice in 1922 gave negative results for cane sugar.

The presence of cane sugar in those varieties in which it only occasionally occurs is in some degree, though not absolutely, correlated with immaturity. Nineteen hundred and twenty was a year of very unfavorable weather conditions during the ripening period—little rain but much cool, foggy weather. In Campbell Early, Catawba, Goethe, Isabella, Jefferson, King Philip, Lady, Noah, Rommel, Salem, Ulster, Vergennes, Wilder, and Worden the cane-sugar content found in 1920 was in each case the maximum found for the variety. In a number of these—Campbell Early, Goethe, King Philip, Rommel, Salem, Ulster, Vergennes and Wilder—the total sugar content is comparatively low as compared with other years. In Salem, Wilder, and Worden acid content is also high. In this group it would appear that unfavorable conditions for photosynthetic activity had resulted in low sugar content and had been accompanied by a partial failure of conversion of cane sugar into hexoses during the ripening period. On the other hand no cane sugar was found in Brilliant, Diamond, Elvira, Gaertner, Merrimac, and Woodruff, although all these varieties failed to develop full normal color or to mature properly, as is further indicated by their low total sugar content. To further complicate the case, 1923 was a year of unusually favorable seasonal conditions in which a number of varieties which occasionally fail to fully mature were able to do so, yet seven varieties (Brighton, Canada, Centennial, Delaware, Dutchess, Early Daisy, and Early Victor), unquestionably fully ripe, showed the presence of cane sugar in that year and at no other time, and the quantity present in a number of others (Agawam, Clinton, Concord, Dakota, "Dutchess Seedling," Eaton, Eumelan, Hartford, Ives, Lenoir, Niagara, and Pocklington) equaled or exceeded that found in other years. Consequently the presence of cane sugar can not be considered as positive proof of the immaturity of the fruit, as a larger number of varieties showed its presence under the exceptionally favorable conditions for ripening in 1923 than in any other year. Nor can it be definitely correlated with any other factor, such as temperature, insolation, water supply, or load of fruit on the vines.

ACID CONTENT

Examination of Tables III and IV will at once show that varieties differ greatly in the amount of fluctuation in acid content occurring from year to year when grown side by side over a number of years. Thus Brighton, Brilliant, Canada, Diamond, Delaware, Eumelan, Herbemont, Lindley, Martha, Niagara, and Noah show a range of variation of less than 0.30 per cent during the period, while Agawam, Clevener, Diogenes, Isabella, Ives, Lenoir, Montefiore, and Norton have a range of 0.60 per cent or more. Yet both groups contain midseason and late-maturing varieties and neither can be said to have an advantage in adaptation to the soil or locality. The fact that the experimental period included exceptionally favorable and decidedly unfavorable years with intermediate conditions has probably resulted in the inclusion in the data of the extremes likely to be encountered in this particular region in a long period of years. Comparison with the data obtained by Alwood in the study of grapes from a number of States indicates that the fluctuation in acid content of a variety grown year after year in the same vineyard, even with very wide differences in seasonal conditions, are much narrower than those encountered in the same variety grown over a wide area with a diversity of soils and cultural conditions.

The extent to which variations in acid and sugar content in the grape are related has been the subject of considerable discussion in the European literature of wine making under the title of "Acid-sugar ratio." The value of a grape variety for wine making depends to a considerable degree upon this ratio, a high acid content resulting in a sour wine. Consequently varieties which were characterized by a fairly constant ratio, low in acid and high in sugar, were most desired, while those which showed widely fluctuating ratios or were high in acidity were less valuable. Alwood in his various publications has devoted some attention to the acid-sugar ratio in American grapes from the same point of view. Very little attention has been given to the question whether sugar and acid con-

tent in a given variety fluctuate entirely independently or with some degree of correlation. Müller-Thurgau (*48*) regarded the increase of sugar and reduction of acidity in ripening fruits not as inseparably related processes but as influenced by conditions such as temperature, water, and nutrient supply, leaf area, and size of crop on the vines, which influence the rate of metabolic processes. Kelhofer (*38*) published a graph summarizing the records for sugar and acid content of Raüchlings grapes for the 14 years 1891–1904, inclusive. Years of maximum acid content were also years of minimum acid content, and vice versa. Years of maximum sugar content for the period in a large number of varieties. These years were also characterized by minimum acid content for the period in a large number of varieties. On the other hand, 1922 and 1920 were years of low sugar content, very few varieties reaching their maximum or next to maximum sugar, and both years are characterized by the almost total absence of low acid readings and the large number of maximum or next to maximum figures found. Furthermore, 1921 is notable for the absence of either very high or very low figures for either constituent. The general conclusion to be drawn from the graph is that a

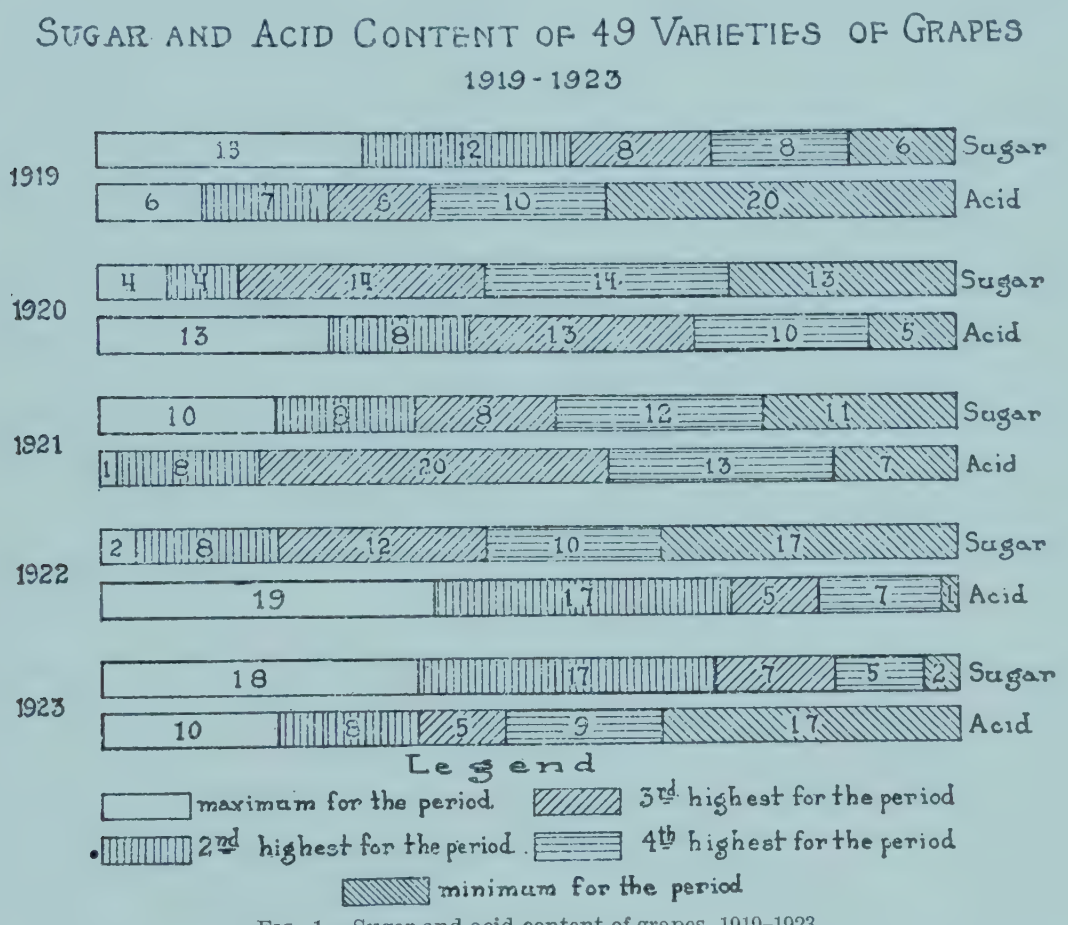

FIG. 1.—Sugar and acid content of grapes, 1919–1923

medium or average sugar content were years of medium acid content. No American studies upon this matter have been found in the literature. It is very important that the facts upon this point be known and understood, since the palatability of an unfermented juice, like that of a wine, depends in no small degree upon the sugar-acid ratio. The results of the present study, in so far as they bear upon the acid-sugar relation, are brought together graphically in Figure 1. From an inspection of the graph it will be immediately evident that 1923 and 1919 were years characterized by maximum or next to year of high sugar content is also one in which acidity is low, and conversely that a year which is markedly low in sugar content is one of more than average acidity. This is very clearly the general situation. There are a number of exceptions: Maximum acid and maximum sugar occur together in Catawba, Franklin, Herbemont, Isabella, Jefferson, and Noah in 1923, in Berckman and Cynthiana in 1922, in Goethe in 1921, and in Montefiore in 1919. Minimum sugar and minimum acid occur only in Dakota and Herbert in 1919 and in Lampasas and Martha in 1922. There are a large number of cases in

which maximum sugar and minimum acid occur together: Colerain, Concord, Dakota, Elvira, Perkins, Rommel, and Ulster in 1923; Brilliant, Goethe, and Niagara in 1921; Martha in 1920; and Clinton, Eumelan, Lindley, Massasoit, and Wilder in 1919. There are fewer instances of occurrence of minimum sugar and maximum acid together: Canada, Dutchess, Lenoir, Merrimac, and Montefiore in 1922, Barry in 1921, and Noah in 1919 make up the list. It is clear that there is no hard and fast connection between acid and sugar content in the grape, but rather a broadly general relationship. Conditions which favor the production of a crop of high sugar content also favor the reduction of acidity to a low level; conditions which depress the sugar production also tend to maintain acidity at a high level. This point will receive further discussion in a subsequent section.

The maximum and minimum acid-sugar ratios occurring in each of 49 varieties during the five years are tabulated in Table V. There is added for purposes of comparison an average acid-sugar ratio for each variety obtained by taking the mean of the yearly ratio for five years. By reason of the fact that the experimental period contained exceptionally unfavorable as well as unusually favorable crop years, the results probably present as wide variations as would be encountered at Vineland in a long series of years. Owing to the inclusion of the unfavorable seasons and differences in the methods of sampling the fruit and expressing the juice, and for perhaps other reasons, the average acid-sugar ratios differ materially from those reported by Alwood. The differences are all in the direction of a higher ratio of acid to sugar, as would be anticipated from the inclusion of two years characterized by low sugar and high acid content.

The varieties studied may be grouped into three groups on the basis of their average acid-sugar ratios. These are a high acid group, consisting of juices whose proportion of acid to sugar is so high as to make them too sharp for beverage use; a balanced or medium group, in which the proportions are such as make the fresh juice agreeable and palatable; and a subacid group, in which the acid content is so low as to make the juice too insipidly sweet to be acceptable as a beverage.

The definition of the exact limits of these groups is a matter of some difficulty. At present Concord is practically the only generally available, commercial grape juice, and it is logical to assume that its acid-sugar ratio is that which most people find agreeable and properly balanced, else it could not have attained its present popularity. It may therefore be made to serve as a starting point in a classification of juices with respect to beverage quality. Hartmann and Tolman (*27*) collected and analyzed 104 hot-pressed commercial Concord grape juices representing the principal producing districts. Their analyses may be assumed to fairly represent the juices available in the markets. The average sugar content for the 104 samples was 15.31 per cent, the average total acidity calculated as tartaric was 1.01 per cent, giving an acid-sugar ratio of 1:15.1. The maximum ratio found was 1:10.7, in a sample having 11.66 per cent of sugar and 1.09 per cent acid. The minimum was 1:19 in a sample

TABLE V.—*Maximum and minimum acid-sugar ratio*

	Maximum	Minimum	Average
Agawam	1:12.1	1:22.5	1:17.8
Barry	1: 9.7	1:16.2	1:12.5
Berckman	1:12.1	1:15.5	1:13.6
Brighton	1:23.8	1:40.5	1:29.4
Brilliant	1:18.8	1:31.4	1:25.4
Canada	1:11.2	1:18.3	1:15.7
Catawba	1:15.3	1:22.4	1:16.1
Clinton	1:10.2	1:14.7	1:11.9
Clevener	1: 6.2	1:10.8	1: 8.9
Colerain	1:13.6	1:26.2	1:20.0
Concord	1:10.9	1:19.8	1:15.7
Cynthiana	1:10.2	1:14.8	1:13.5
Dakota	1:10.0	1:15.6	1:12.2
Diamond	1:11.9	1:18.2	1:15.1
Diana	1:16.7	1:24.7	1:19.7
Diogenes	1: 8.2	1:16.0	1:11.2
Delaware	1:20.5	1:32.8	1:24.8
Dutchess	1:16.4	1:32.3	1:24.8
Elvira	1:11.5	1:19.2	1:14.6
Eumelan	1:23.7	1:41.7	1:29.4
Franklin	1: 7.5	1:11.7	1: 9.5
Goethe	1:14.6	1:20.9	1:17.3
Herbemont	1:11.7	1:13.6	1:12.7
Herbert	1:14.6	1:21.1	1:17.9
Isabella	1:13.9	1:21.3	1:16.7
Ives	1: 9.1	1:28.0	1:19.1
Jefferson	1:16.4	1:24.4	1:20.2
Lampasas	1:15.3	1:23.6	1:19.6
Lenoir	1: 6.6	1:11.4	1: 9.5
Lindley	1:21.1	1:37.1	1:25.6
Lucile	1:18.4	1:29.1	1:22.2
Martha	1:25.8	1:34.7	1:30.9
Massasoit	1:12.5	1:25.7	1:18.9
Merrimac	1:14.9	1:23.3	1:19.7
Missouri Riesling	1:13.7	1:17.8	1:16.2
Montefiore	1:14.4	1:31.9	1:23.7
Nectar	1:10.9	1:24.2	1:18.1
Niagara	1:21.6	1:28.0	1:23.9
Noah	1:11.0	1:16.6	1:14.5
Norton	1:10.6	1:17.6	1:14.4
Perkins	1:14.8	1:26.0	1:19.7
Pocklington	1:12.0	1:23.6	1:19.5
Rommel	1:13.9	1:22.0	1:17.4
Salem	1:15.4	1:27.6	1:21.0
Ulster	1:15.8	1:26.7	1:20.2
Vergennes	1:17.1	1:25.5	1:20.5
Wilder	1:11.5	1:35.1	1:20.8
Woodruff	1:17.9	1:24.1	1:20.7
Worden	1:10.7	1:25.6	1:15.8

with 17.31 per cent sugar and 0.91 per cent acid. With these exceptions, the juices studied by Hartmann and Tolman had ratios between 1:12.5 and 1:17.5, with 1:15 as the mean. If Hartmann and Tolman's results are fairly representative of commercial Concord grape juice, as their number and distribution would indicate, it may be assumed that the public finds juices having such a range in acid-sugar ratios acceptable for beverage purposes in so far as these constituents are concerned, while those above or below these limits fall into the high acid and subacid groups, respectively. Of course, the palatability of a juice does not depend wholly upon the acid-sugar ratio, but this is a factor of outstanding importance.

Carrying out the arrangement of the varieties into groups upon this basis, we have the three groups constituted as follows:

HIGH-ACID GROUP (acid-sugar ratio 1:12 or higher): Barry, Clinton, Clevener, Cynthiana, Dakota, Diogenes, Franklin, Herbemont, and Lenoir.

MEDIUM OR BALANCED GROUP (acid-sugar ratio about 1:15): Berckman, Canada, Catawba, Concord, Diamond, Elvira, Goethe, Isabella, Ives, Missouri Riesling, Nectar, Noah, Norton, and Worden.

SUBACID GROUP (acid-sugar ratio 1:17.5 or less): Agawam, Brighton, Brilliant, Colerain, Diana, Delaware, Dutchess, Eumelan, Herbert, Jefferson, Lampasas, Lindley, Lucile, Martha, Massasoit, Merrimac, Montefiore, Niagara, Perkins, Pocklington, Salem, Ulster, Vergennes, Wilder, and Woodruff.

Some varieties do not fit very closely into this classification because of variations in acid-sugar ratio which are considerably wider than the limits of any one group. Examples are Agawam, Barry, Colerain, Diogenes, Ives, and Wilder. These are varieties which show a wide variation in acid or in sugar content or both, as may be seen by reference to the analytical data in Table III, with the result that the ratio in some years stands apart from the others. Such varieties have been placed in the groups to which the results for the remaining years, together with the average, would place them. Canada, Montefiore, and Perkins in one year (1922) had crops of very high acidity and minimum sugar with resulting ratios materially higher than for other years. The very unfavorable weather conditions prevailing during the ripening period in 1922 affected several other late-maturing varieties in the same way. The widest variations in ratio are found in the subacid group, Brighton, Brilliant, Eumelan, Lindley, and Wilder showing especially wide ranges. Two varieties, Noah and Norton, stand on the border line between the high-acid and the balanced groups.

Broadly speaking, the fact that the acid-sugar ratio is as constant as the figures show it to be when the crops were produced under the variety of seasonal conditions encountered at Vineland in 1919–1923 shows conclusively that knowledge as to the ratio for a variety is of material assistance in indicating its possibilities as a source of beverage juice.

TOTAL ASTRINGENCY, OR TANNIN, CONTENT

The suitability of a fruit juice for beverage purposes depends not only upon the amounts of sugar and acid it contains but also upon the amounts of astringent constituents present. A palatable beverage juice must have a certain fairly definite balance or proportion between the three constituents—sugar, acid, and tannin. If the tannin is deficient in amount the juice will be lacking in sprightliness and agreeable aftertaste; if it be in excess the juice will be harsh and astringent with a suggestion of bitterness. The insipid character of the juices of many of our choicest table varieties of grapes and the harshness and lack of palatability of the juices of most wild grapes are instances of these extremes, while Concord juice is an example of a fairly well balanced juice. The amount of astringent material present is as important as the sugar-acid ratio in determining the value of a grape juice for beverage purposes.

While a considerable number of methods for the determination of total astringency and astringent nontannins in fruit juices have been proposed, none of them has displaced the Loewenthal-Proctor method, which remains essentially as described by Neubauer in 1872 (*50*) with the difference that powdered kaolin is substituted for bone black in filtering off the gelatin-tannin precipitate. For quantitative purposes this method is faulty in several respects. The chief defect is that all substances which reduce $KMnO_4$ are included in readings as tannins, either wholly or in part. The products of the reaction of tannin with gelatin are variable and the precipitation is a colloidal one which varies with conditions, as Wood (*62*) has shown. The filtration of the tannin-gelatin precipitate with kaolin involves a variable error, since the red pigment of some deeply colored juices is very strongly adsorbed by the filter paper used and can not be wholly re-

moved by prolonged washing with water. The calculation of the non-tannin astringency to grams per 1,000 c. c. by the use of the factor used for tannin, involves a large error, as the substances making up the astringent nontannins are highly diverse in character. It has been known since the work of Neubauer (*50*) in 1872 that the pigment of a certain red wine, studied by him, when prepared in a fairly pure state, had only 30 per cent the reducing power of pure tannin (0.00754 gram of Neubauer's preparation equalled 0.002296 gram tannin in reducing power for $KMnO_4$), while Kelhofer (*39*) found that when he titrated purified pear tannin with $KMnO_4$ the results indicated only 64 per cent of the weight of tannin actually employed in the determinations. For these reasons the results obtained when the method is employed for a large number of juices which vary considerably in nature and amount of coloring matter and other oxidizable nontannins present are at best only roughly comparative.

A study of the literature on methods of determination of tannin in fruit juices was undertaken in the hope that a method could be found which would differentiate somewhat more accurately between tannins and nontannins and which could be used in work with fresh juices under rather primitive field conditions. A review of the summaries by Nierenstein (*51*, *52*) and Czapek (*18*), and of the work of Spiers (*55*), Laborde (*41*), Fresenius, and Grünhut (*22*), Meyer (*47*), and others indicated that there is no method which can be depended upon for quantitative results which can be used in field work. Since the results reported in the literature are expressed in terms of the Loewenthal-Schroeder method as modified by Proctor, its employment would permit direct comparison of results and it was therefore employed for the field work. Another consideration which led to the abandonment of any attempt to obtain anything more than roughly comparative results upon the fresh juices is the very large and rapid change in soluble astringent constituents which freshly pressed juices undergo upon exposure to the air. As a consequence of this alteration, which has been recorded by Lindet (*43*), Hotter (*32*, *33*), and studied especially by Kelhofer (*35*, *36*, *37*, *39*), the tannin content of a freshly pressed fruit juice is a maximum which decreases as the juice passes through the processes of bottling and pasteurization to reach a stationary point sometime after pasteurization. Therefore such determinations do not give any indication as to the degree of astringency a given juice will possess when it reaches the consumer, if prepared in the usual way. Their chief interest is through the indications such determinations may give as to the nature and extent of the effect of seasonal conditions upon the content of astringent constituents. It is a fact of considerable practical significance that juices which have been clarified by filtration with diatomaceous earth by the methods described by the writer (*17*), do not undergo loss of astringency upon pasteurization. This method of filtration therefore has the merit, not fully appreciated when the paper referred to was prepared for publication, that it not only gives permanently clear filtrates but that it also preserves the juices from subsequent alteration in their sugar-acid-tannin ratios. A detailed study of the chemical changes in apple and grape juices as a result of pasteurization is now being made as one phase of the investigations upon fruit juices in progress in this laboratory and will be published separately. A discussion of the effects of diatomaceous earth filtration as related to pasteurization will be included in that paper, reference being made here by reason of its practical importance.

Tannin determinations upon fresh juices were not made in 1919 and 1920, so comparisons for only three seasons are possible. The data obtained in 1921, 1922, and 1923 are presented in Table III.

There are in the literature very few similar determinations with which comparisons may be made. Hartmann and Tolman determined tannin and coloring matter in the series of Concord juices studied by them, but these were hot-pressed juices, and the determinations were made after pasteurization, and hence are not comparable. Noyes, King, and Martsolf (*53*) made tannin determinations on Concord grapes throughout the harvest period, and Alwood and his collaborators (*2*) made similar determinations in their studies of ripening in Catawba, Clinton, Delaware, Ives, Norton, and Concord. The results in both these pieces of work indicate that the tannin and coloring matter fluctuate very irregularly throughout the ripening and harvest period, without definitely increasing or decreasing after the berries have begun to color. Tannin determi-

nations were not made by Alwood in his more extensive studies of grapes from the eastern districts, so his results give no information as to the variation in these constituents from year to year or when grown in various localities.

The outstanding fact brought out by the determinations of astringency is that there is very considerable fluctuation in total astringency in all varieties, Cynthiana, Franklin, Martha, Norton, and Wilder being exceptional in that they are comparatively constant. 1923 was a year of notably low total astringency, 42 varieties showing the minimum amounts while only two, Clevener and Wilder, had their highest astringency. 1922 was a year of markedly general high astringency, 35 varieties showing the maximum for the period in that year, while only four varieties—Catawba, Clevener, Diogenes, and Franklin—had their minimum. 1921 occupied an intermediate position, 11 varieties showing maximum, while only three—Isabella, Lampasas, and Wilder—have the minimum.

While the records of the total astringency at pressing cover only three years and are therefore insufficient to warrant extended generalizations, it may be pointed out that the results show a parallelism with the acid determinations. The comparisons for acidity cover five years' results, those for astringency only three, but the tendency to fluctuate together is clear. 1923 was a year characterized by minimum or next-to-minimum acid content in more than half the varieties, while more than four-fifths show the minimum in total astringency. 1922 was a year of high acidity, 36 varieties out of 49 showing maximum or next-to-maximum acid content for the five-year period, while 35 had the maximum total astringency encountered in the three-year period. In 1921 the acidity for the great majority of varieties was intermediate, few varieties appearing at either extreme, while 32 have total astringency figures intermediate between those of 1922 and 1923.

As might be anticipated when the diversity of the substances determined as astringent nontannins is considered, the part contributed by these to the total astringency reading fluctuates considerably from year to year in most varieties. Contrary to expectation, the fluctuations in nontannin astringency found in white varieties, such as Colerain, Diamond, Dutchess, Elvira, Niagara, Noah, and Pocklington, are proportionately as wide as those in heavily pigmented varieties, though the absolute amounts are considerably smaller. Broadly speaking, total astringency and true tannins rise or fall together, 33 varieties having maximum tannin in 1922, while 20 had minimum tannin in 1923. Twenty-seven had maximum astringent nontannins in 1922, while 44 had minimum astringent nontannins in 1923, so it seems that the amount of these constituents rises or falls with the total astringency but not necessarily to the same degree. While determinations for a 3-year period do not warrant dogmatic conclusions, it would appear from the data that there is a considerable degree of correlation between acidity, total astringency, and tannins and astringent nontannins, and that all these factors respond to seasonal conditions in like manner but in varying degree.

CLIMATIC CONDITIONS AT VINELAND FOR PERIOD OF EXPERIMENT

The Weather Bureau has maintained a station in Vineland since 1868, and the data as to rainfall, sunshine, and temperature for the period have been taken from the published reports for that station in Climatological Data: New Jersey section for the years 1917–1923, inclusive. More detailed records of the stations at Trenton and Atlantic City, N. J., and Philadelphia, Pa., as published in the Monthly Meteorological Summary, Form No. 1030, by each of these stations, have been furnished through the kindness of the meterologists of these stations; and a transcript of the record kept at Wilmington, Del., was supplied by the street commissioner of that city. Comparative study of the detailed records at these stations has been of assistance in interpreting the less detailed Vineland records.

The first four years of the period covered by the work has been characterized by the meterologist for New Jersey (*60*) in the following words:

> In the State, as a whole, there has been from the year 1917 to date (November, 1922) a steadily increasing deficiency of precipitation. The years 1919 and 1920 were slightly above normal in precipitation, but all the other years, or almost any one of them, more than made up for those two years of excess. The lack of water supply, then, must be attributed to more than the deficient rainfall of the past few months.

This statement might with equal truth have been written a year later, and with specific reference to condi-

tions at Vineland, as the rainfall record at the end of November, 1923, for that station stands as follows:

	Inches.
Deficiency for 1917	−5.10
Deficiency for 1918	−7.94
Excess for 1919	12.61
Deficiency for 1920	−.44
Deficiency for 1921	−10.07
Deficiency for 1922	−.86
Deficiency for 11 months of 1923	−3.96
Accumulated deficiency [10]	15.76

The progressive accumulation of a deficiency of this amount had a progressively increasing effect upon the ground water level, as was evidenced in the last three years of the work by the failure during the summer and autumn months of many of the shallow wells in the vicinity of the vineyard. Water is usually obtained everywhere in the area by wells driven to a depth of 15 to 25 feet, but many of the shallow wells failed entirely in the autumn of 1921 and yielded considerably reduced supplies in 1922 and 1923.

The year 1917 was characterized by an average mean temperature 1.9° below the 49-year average, the mean temperature for the separate months exceeding the average only in January, March, April, and August. May was markedly cool, 5.7° below the average; June and July were normal; August 1.6° above normal; and the remaining months of the year 3.1°, 2.6°, 1.9°, and 5.4° below the average.

The rainfall, while only 5.10 inches below the average annual precipitation, was so distributed as to result in a degree of drought during the growing season which affected the yields of many crops. An excess of 2.52 inches in March did not make up the shortage for the preceding months, there was a deficiency of 1.55 inches in May, 3.94 inches in August, and 0.93 inch in September, the other summer months showing normal or a few hundredths more than normal precipitation. An excess of 5.26 inches in October fell mainly toward the end of the month, too late to directly affect crops. The six "growing months," April to September, inclusive, received 6.10 inches less than the normal precipitation for the period, so that the grape crop was grown with a material shortage of water and ripened in a period of abnormally cool dry weather.

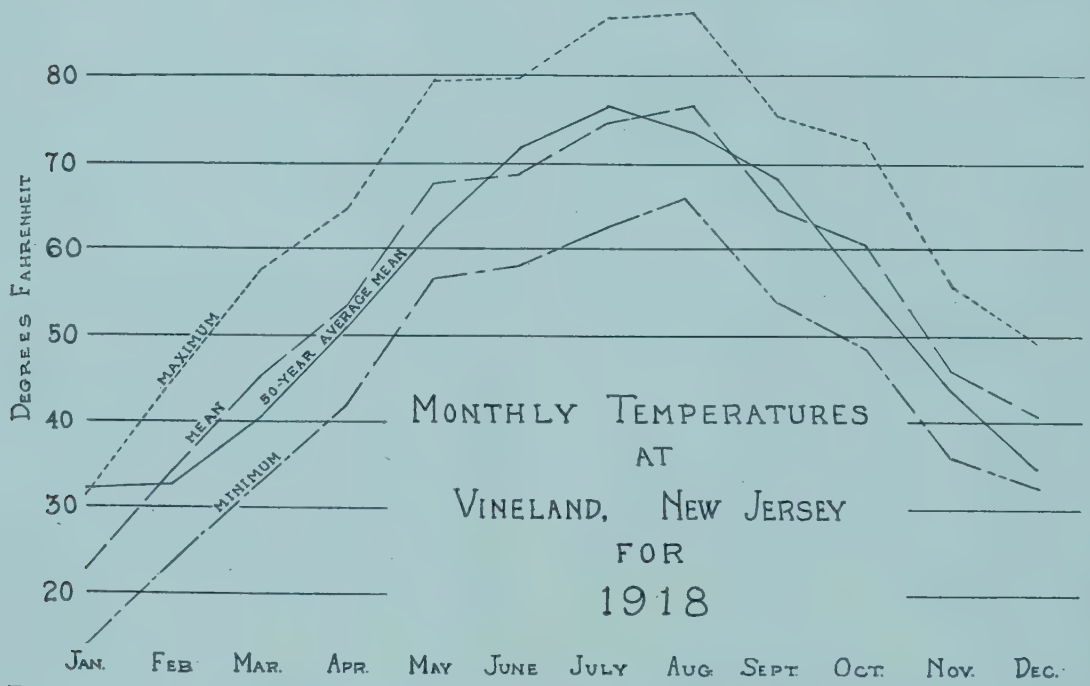

FIG. 2.—Maximum, minimum, and mean monthly temperatures at Vineland, N. J., in 1918, with average mean temperature for 49-year period for comparison

CLIMATIC RECORD FOR 1918

The climatic conditions for 1918 are graphically presented in Figures 2 and 3. Figures showing percentages of possible sunshine at Vineland are not published separately, but a record of days classified as "clear" (having 0 to 30 per cent of cloudiness during hours of possible sunshine), "partly cloudy" (having 40 to 70 per cent of cloudiness), and "cloudy" (having 80 to 100 per cent of cloudiness) is kept, and these data

[10] These figures represent a close approximation rather than an exact statement, for the reason that the rainfall records for Vineland are incomplete or missing for March, 1917, September, 1918, March, 1919, July and August, 1920, and February, 1922. Figures for the stations at Bridgeton, 12½ miles southwest of Vineland and Hammonton, 17 miles northeast, have been supplied for the missing months, as the rainfall at these stations closely approximates that occurring at Vineland.

are presented in Figure 3. The detailed data from the adjacent stations at Atlantic City, Philadelphia, and Trenton have been examined, and some general facts adduced therefrom are stated in the subsequent discussions, but the graphs representing sunshine are based on the Vineland data alone. The mean temperature records show that January was a month of exceptionally low temperatures, the coldest on record in the State. From February on through May the mean was consistently well above the average, the last killing frost occurring on May 7. June, July, and September were slightly below, August somewhat above normal, light showers until mid-July, when a period of extreme heat and drought set in. This was broken by rainfall of 6.36 inches on July 31. August was extremely hot with only one wetting rain (0.64 inch) on the 15th. This somewhat accelerated the maturing of the crop. The normal rainfall and cooler weather of September, followed by drought and heat through October, permitted normal ripening of a crop of slightly less than average size. November was mild, dry, and warm, the first killing frost occurring on the 7th, and December was normal in rainfall but exceptionally warm and without snow. The year was one which was

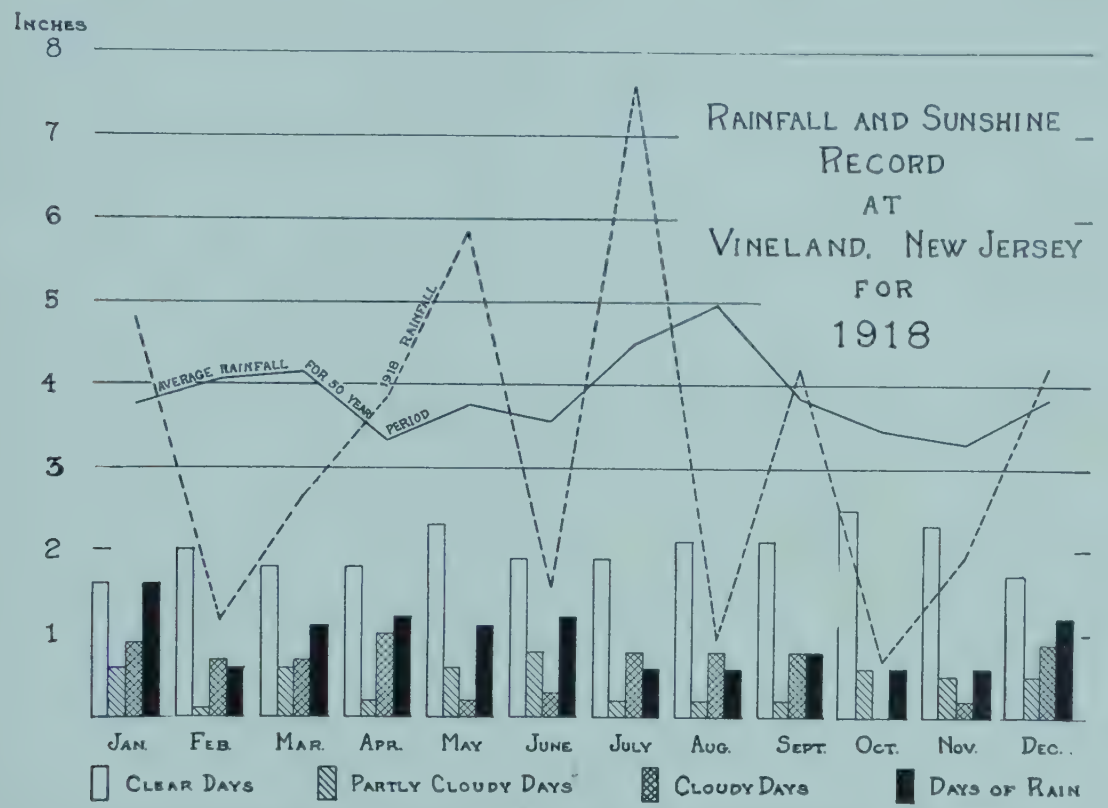

FIG. 3.—Rainfall and sunshine at Vineland, N. J., in 1918, with 49-year average for comparison. (All days on which a trace, 0.01 inch or less, was recorded are included; hence, some days recorded as clear are also included under days of rainfall)

and warm mild weather was continuous from October 1 through the remainder of the year, the first killing frost occurring on November 7.

The outstanding features of the precipitation for the year was its marked deficiency (7.94 inches), its irregular distribution, and the infrequency of prolonged periods of cloudy, showery weather. The vines began to grow early in the season and the conditions during the blooming period were favorable to the setting of a crop of fruit of normal size on almost all varieties. Pollination of a few varieties was somewhat interfered with by rain on May 9. The low rainfall of June was accompanied by cool weather with occasional considerably deficient in soil moisture throughout the growing season but which supplied conditions for maximum photosynthetic activity through an unusual percentage of sunshine well distributed over the entire growing season. That this characterization of the condition for photosynthesis is justified will be at once evident from a comparison of Figure 3, showing number of clear days per month, with the graphs presenting like data for subsequent years. The seven months, March to September, inclusive, which cover the period from resumption of growth to maturity of the fruit, had 18 to 23 days each which were reported as clear, a total of 141 days for the seven months.

This indicates a degree of freedom from prolonged periods of cloudy, showery weather and fogs, quite exceptional for spring and summer at Vineland.

This statement of the crop conditions for the two years preceding the beginning of the chemical studies may serve as a background for that work and assist in the interpretation of the data obtained in subsequent years.

Climatic Record for 1919

The outstanding features of the year 1919 are the mildness of the winter, the consistently above normal mean temperatures from January to June and from October to the end of the year (fig. 4), the large excess of precipitation (12.61 inches) over the average, and the occurrence of almost one-fourth of the total rainfall in July and August (fig. 5).

Rainfall was slightly below normal for the winter and spring months but was excessive in May, which had three weeks of almost constantly cloudy, warm weather with rain on 13 days, 3.47 inches being recorded on the 10th. June was normal in temperature and sunshine but deficient in rainfall. July and August were marked by almost continuous cloudiness. From July 6 to 24 there were 14 days when it rained. August had 11 days rain. A heavy gale of wind, accompanied by 5.61 inches of rain, on August 14 did considerable damage to vines and fruit. The total rainfall for the two months, 13.97 inches, was 4.59 inches in excess of the normal, and the low sunshine for the whole period was unfavorable to the crop. Ripening of the earlier varieties was considerably retarded by cool, cloudy weather, with light showers during the first half of September, after which warm dry weather, which extended with increasingly abnormal high temperature through October resulted in rather rapid ripening. November was dry and warm, the first killing frost occurring on the 9th, and December was unusually cold, with more than the usual amount of snow.

To sum up the climatic conditions for 1919 as they are related to the production of the crop, the deficiency in soil moisture carried over from 1918 was continued until July, when the crop was well advanced, and was not wholly made up until the middle of August. The period June to September was one of practically normal mean temperatures and normal percentages of possible sunshine, while the early spring months and the ripening period had a slight excess of both temperature and sunshine. As the vines bore a slightly smaller crop than usual, it would be a justifiable presumption that the fruit would show no marked departures from normal in its composition. The data for clear, partly cloudy, and cloudy days presented in Figure 5 show that the whole period of development and maturity of the crop was characterized by a predominance of clear, sunshiny days, varying in number from 18 to 22 per month. In number of clear days during these

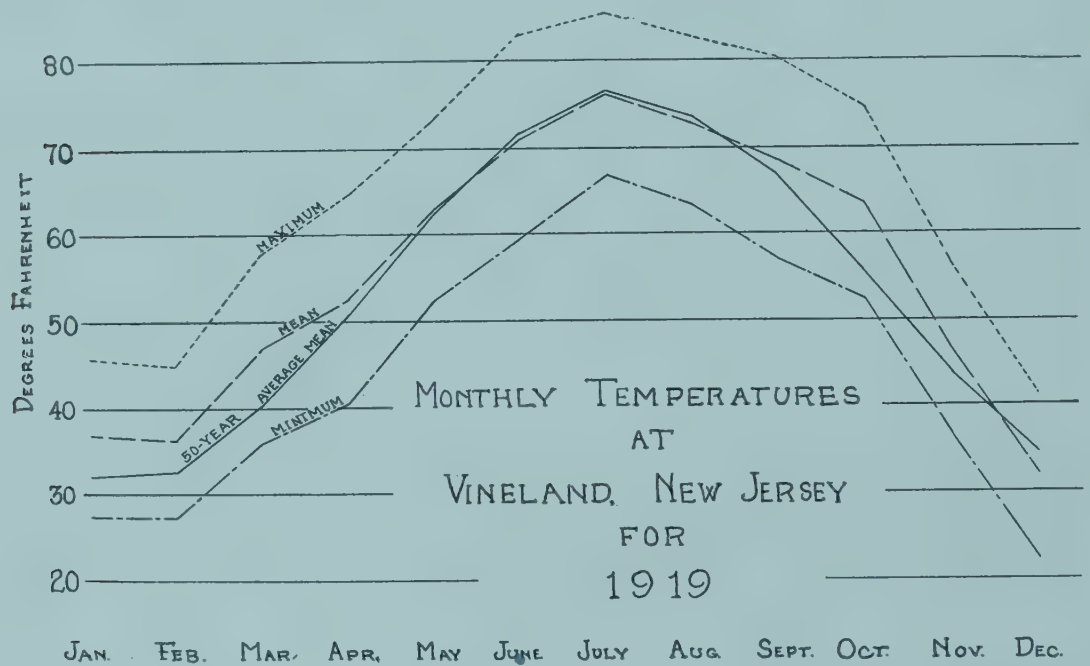

FIG. 4.—Maximum, minimum, and mean temperatures in 1919 at Vineland, N. J., with 50-year average mean for comparison

months, 1919 is first of the five years during which sampling of the crop was carried on.

Climatic Record for 1920

The unusually cold weather of December, 1919, continued through January, 1920, but abated somewhat in February. The spring months were slightly above normal, July almost 3° below normal, and all the subsequent months of the year 1 to 5° above the 50-year average (fig. 6). The last killing frost of the spring occurred May 6, but the damage done to grapes was very slight. The rainfall for the year was 0.44 inch less than normal—but was abnormal in distribution—the winter and early spring months having a little less than usual, with an excess of 4 inches in June, a deficiency of 80 per cent in October, and an excess in November and December. It is evident from inspection of Figure 7, in comparison with Figures 3 and 5, that the percentage of sunshine in the growing months of 1920 was considerably less than that for the same period in 1918 and 1919. Such comparison is facilitated by reference to Table VI, which presents the data for six years, 1918–1923, for the period March–September, inclusive.[11] The number of clear days reported for this period in 1918 and 1919 was 141 and 134, respectively, as against 110 for 1920 (fig. 7), or, expressed as percentages of the total number of days in the period, the clear days were 65.8, 62.6, and 51.26 per cent of the possible for the three years 1918, 1919, and 1920. The differences between 1919 and 1920 are great enough to be of considerable significance in determining the amount of photosynthetic activity for the season. To arrive at any accurate comparison however, the days recorded as partly cloudy must also be evaluated. The term "partly cloudy," as used in the weather reports, is applied to any day which has 40 to 70 per cent of its possible sunshine prevented by clouds. For comparative purposes, some value must be assigned to these days, as soon as photosynthetic activity occurred thereon. If we arbitrarily assume that where large numbers are concerned, partly cloudy days have on the average 50 per cent of sunshine, and add to the number of clear days one-half the number of

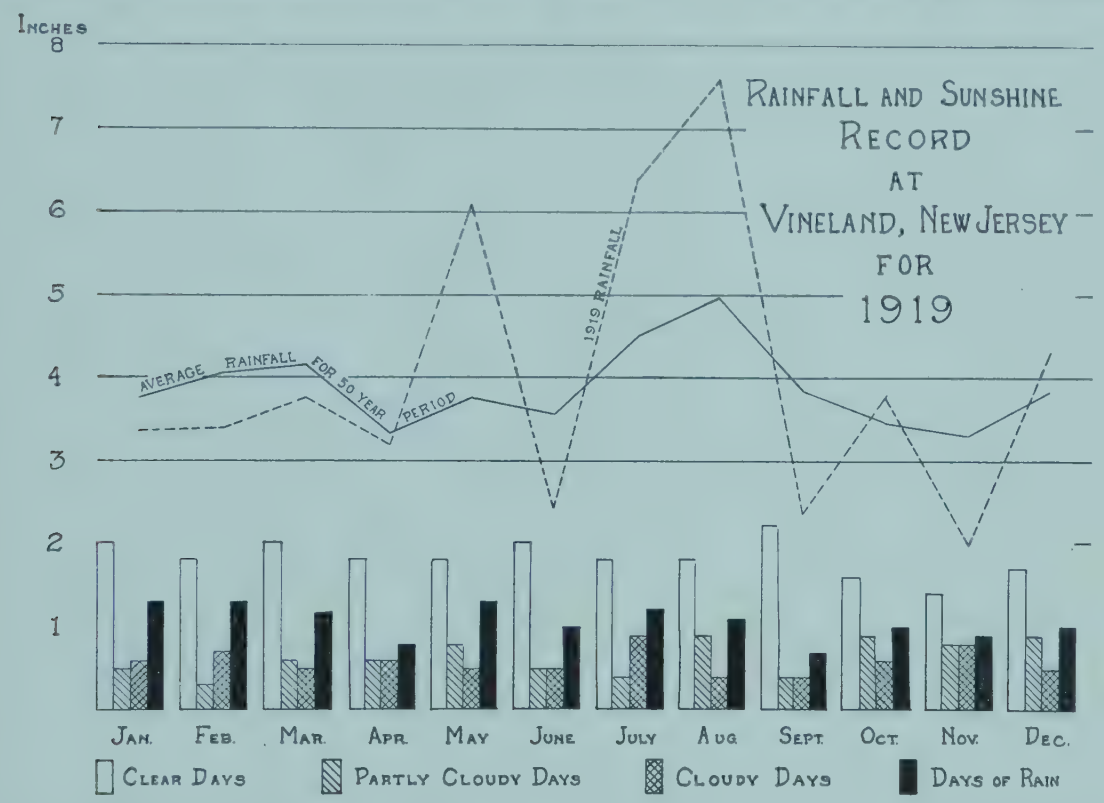

Fig. 5.—Rainfall and sunshine at Vineland, N. J., in 1919, with 50-year average for comparison. (All days showing a trace, 0.1 inch or less, are included. Some inconsistencies will be noted in the graph, the days of rain plus the number of clear days in some instances exceeding by one or two the number of days in the month. In some instances this is due to incompleteness of the Vineland records, necessitating substitution of data from the nearest station; in others to inconsistency in the Vineland records themselves as, days on which a trace of rain fell were reported as being clear)

[11] This period is chosen for comparative purposes for the reason that grape harvest is well advanced and often practically complete by the end of September, only a few late maturing varieties remaining to be gathered after October 1 in normal seasons.

recorded as partly cloudy, we have 158, 155, and 145 days of the 214-day period as days of sunshine in the three years. Expressed as percentages, these equal 73.8, 72.4, and 67.7 per cent of the total number of days in the period. It must be emphasized that in making this comparison an arbitrary valuation has been assigned to the sunshiny hours of partly cloudy days, and that the figures arrived at must not be regarded as exact mathematical statements of what occurred in the three seasons in question. It is clear from the data in Table VI, however, that for the period covered by this work the year 1920 had the smallest number of clear days and the largest number of partly cloudy or wholly cloudy days during the growing season, and that consequently the sunshine received was minimum in amount as compared with the other years of the period. While the fruit of most of the varieties remained on the vines a week to two weeks after the end of September, the season being exceptionally late, this did not materially increase the amount of sunshine received for a reason stated in the next paragraph.

The situation which actually existed during the period September 15 to October 15 is not indicated by the monthly summary of weather data in the graph. The period September 13 to 23 was rainless, and ripening of the earlier varieties proceeded normally. A period of cloudy, sunless weather began on the 24th and continued through the remainder of the month, more than three-fourths of the precipitation for the month occurring on the 26th, 28th, and 29th. While the only precipitation in October occurred on the 28th the period between the 1st and 16th was remarkable for the nightly dense fogs which usually persisted till midafternoon and broke away to recur shortly after nightfall. The temperatures during the nights and forenoons were low, and though there were short periods of sunshine in the afternoon during which a fairly high maximum was attained the ripening of the fruit was slowed down greatly and some varieties did not reach normal coloration. The harvest extended from September 18 to October 17, at least a week longer than in any other year.

The persistence of warm weather through November delayed the appearance of killing frost until November 12. There was an excess of rainfall in both November and December with total absence of snow and the usual December temperatures.

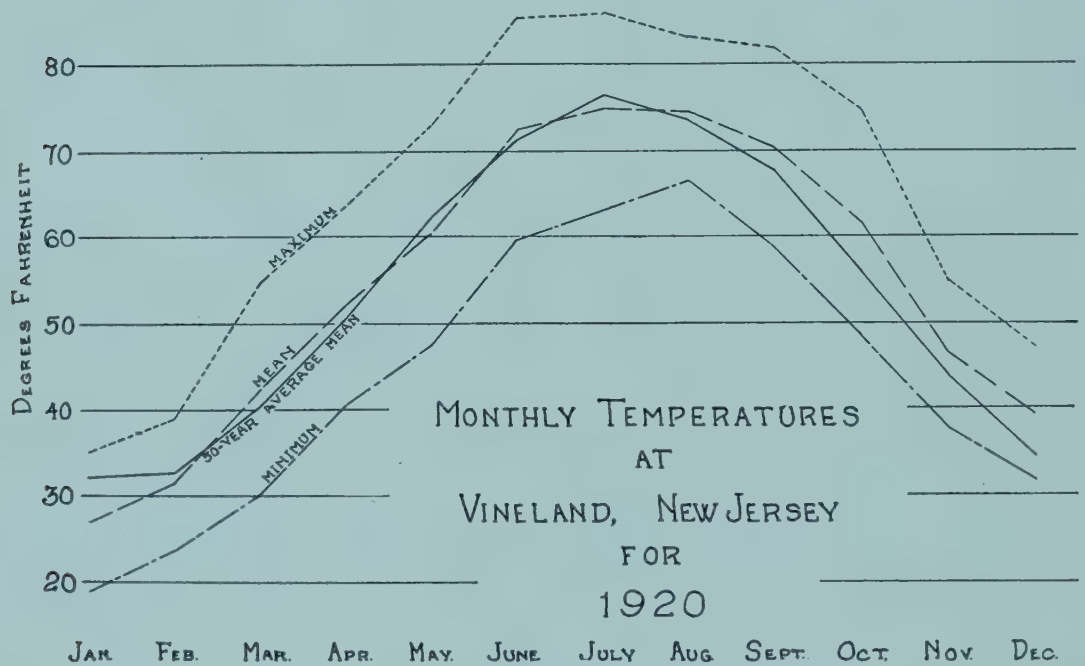

FIG. 6.—Maximum, minimum, and mean temperatures at Vineland, N. J., in 1920, with 50-year average for comparison

CLIMATIC RECORD FOR 1921

The outstanding features of the climatic record for 1921 are the almost total absence of typical New Jersey winter weather and the great deficiency of precipitation throughout the State (10.07 inches at Vineland) (figs. 8 and 9). The mean temperatures at Vineland for the four months January to April, inclusive, were 4.6°, 5.4°, 14.4°, and 8.6°, respectively, above the 50-year average, the minimum temperature recorded in March being 1.7° above the normal mean temperature for the month. This highly abnormal

condition hastened the development of vegetation, with disastrous results to the grape crop. A cold wave of several days duration at the end of March was accompanied by heavy frosts and temperatures of 24° on March 29 and 27° March 30 and April 2. The young shoots on most varieties were at that time 4 to 10 inches long, with the blossom clusters showing plainly on the earlier-blooming varieties. All were killed. On April 11 and 12 a

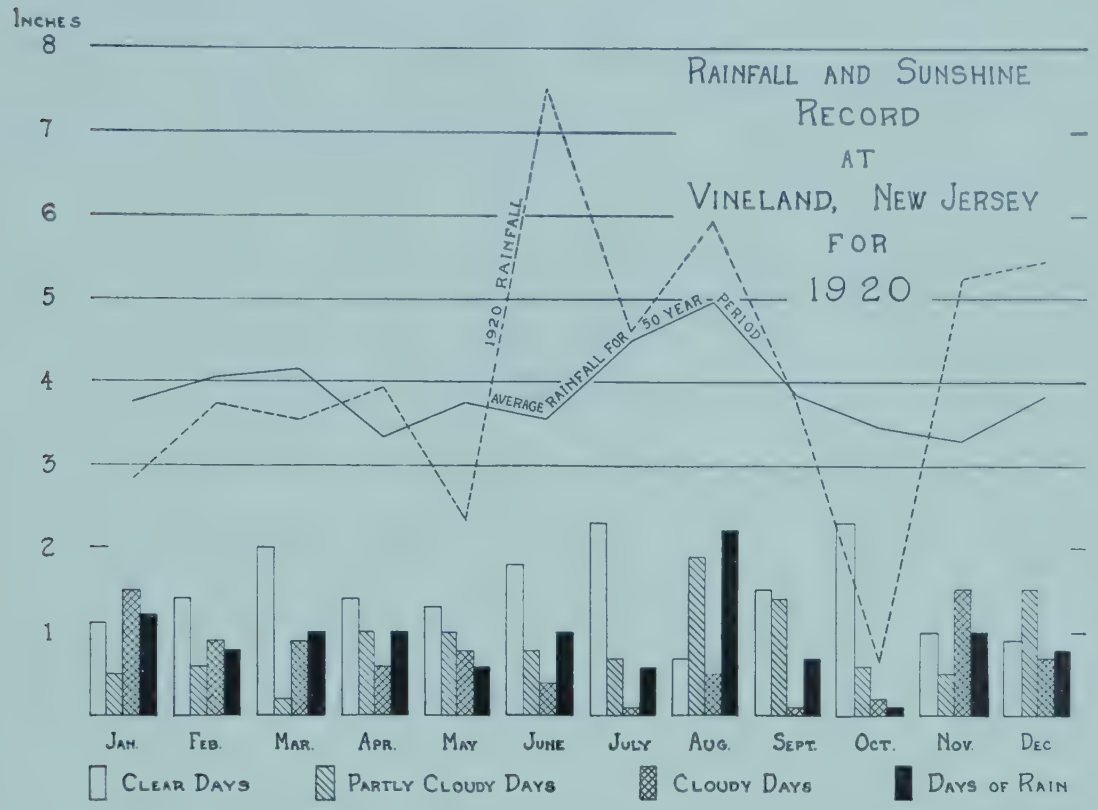

FIG. 7.—Rainfall and sunshine at Vineland, N. J., in 1920, with 50-year average for comparison. (All days on which a trace, 0.1 inch or less, was recorded are included)

TABLE VI.—*Number of days recorded as "clear," "partly cloudy," and "cloudy" at Vineland, March to September, inclusive, 1918 to 1923, inclusive*

	March	April	May	June	July	August	September	Number	Percentages
1918:									
Clear	18	18	23	19	21	21	21	141	65.8
Partly cloudy	6	2	6	8	6	2	2	32	15.0
Cloudy	7	10	2	3	4	8	7	41	19.1
1919:									
Clear	20	18	18	20	18	18	22	134	62.6
Partly cloudy	6	6	8	5	4	9	4	42	19.65
Cloudy	5	6	5	5	9	4	4	38	17.75
1920:									
Clear	20	14	13	18	23	7	15	110	51.26
Partly cloudy	2	10	10	8	7	19	14	70	32.7
Cloudy	9	6	8	4	1	5	1	34	16.0
1921:									
Clear	19	14	14	17	18	22	15	119	55.6
Partly cloudy	9	7	8	10	9	3	8	54	25.2
Cloudy	3	9	9	3	4	6	7	41	19.1
1922:									
Clear	14	18	19	11	19	12	21	114	53.2
Partly cloudy	6	5	6	10	10	10	3	50	23.36
Cloudy	11	7	6	9	2	9	6	50	23.36
1923:									
Clear	16	19	21	21	12	16	15	120	56
Partly cloudy	8	4	6	7	15	10	10	60	28
Cloudy	7	7	4	2	4	5	5	34	16

Percentage of possible clear days: 1918, 73.36, or 158; 1919, 72.45, or 155; 1920, 67.75, or 145; 1921, 68.22, or 146; 1922, 64.95, or 139; 1923, 70.09, or 150.

minimum temperature of 29° was recorded at Vineland. This completed the destruction of the flowering shoots on all except a few varieties in which growth had not advanced far. Thirteen varieties—Agawam, Cynthiana, Franklin, Hartford, Herbemont, Iona, Lenoir, Missouri Riesling, Noah, Norton, "Seibel Hybrid No. 2," Vergennes, and Wilder—suffered little or no apparent injury. With all other varieties there was either entire failure to fruit or considerably smaller than normal crops from buds which were dormant at the time of the freeze (*9*). Unfortunately it was not possible to visit the vineyard at the time of the freeze, so the only information available on the effects of the freeze is that given by the owner and his employees, two of whom were thoroughly familiar with the vineyard through continuous employment therein from the time it was planted. Their statements agree that in practically all varieties two to three weeks were required for development of new growth to the point it had reached at the time of the freeze.

Data on the yield of the various varieties were obtained in detail at the time of harvesting the crop, as it was recognized that reduced yields might materially affect the chemical composition of the crop. This data is presented as percentages of normal crop in Tables III and IV.

The precipitation for the year was very markedly deficient. The winter months were dry. Rainfall equaled the normal in March and very slightly exceeded it in April, after which it remained below normal until the close of the growing season. The months of April to October received only 18.44 inches of rainfall as compared with the normal 27.01 inches for this period, so that the whole period of development and ripening of the crop was marked by a steadily increasing deficiency of water. Severe drought conditions developing in July were partially relieved by the August rains, which were 86.6 per cent of the normal, but drought again became severe in September and October.

The mean temperature, after dropping to normal in May, rose again in June and July considerably above the average, dropping slightly below normal in August, then remaining above normal for the remainder of the year.

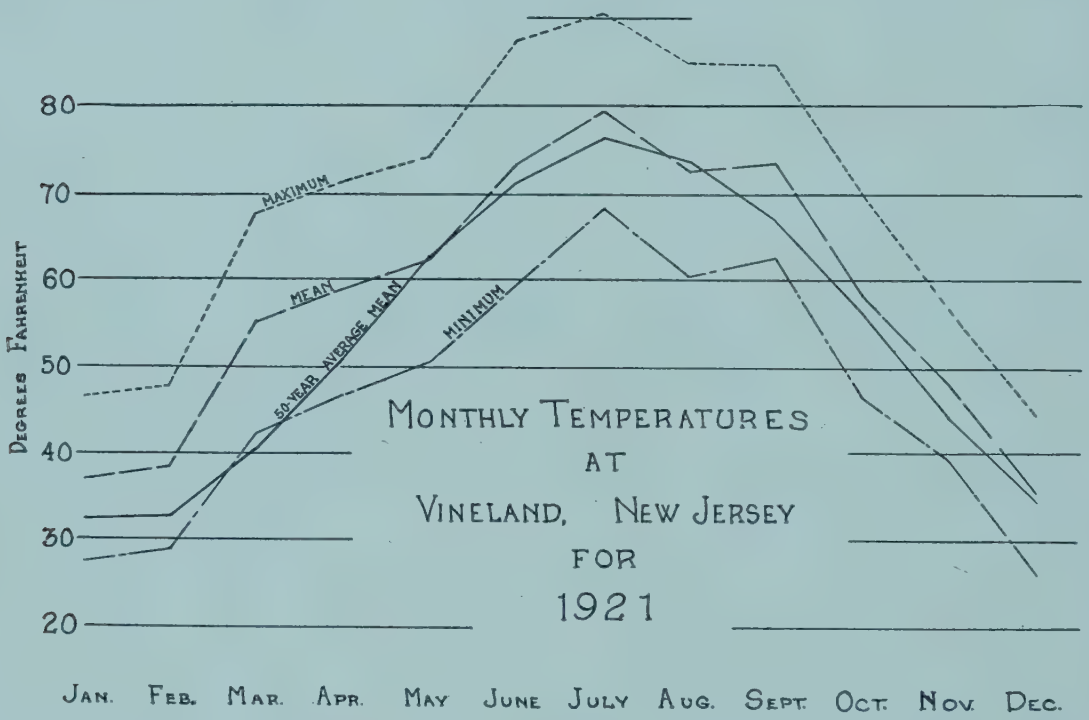

FIG. 8.—Maximum, minimum, and mean monthly temperatures in 1921 at Vineland, N. J., with a 50-year average for comparison

The number of days reported as clear in the months March to September, inclusive, was 119, or 55.6 per cent of the total, as against 110 clear days, or 51.26 per cent of the total, for the year 1920. If we repeat the assumption made in the discussion of the 1920 conditions, that one-half the time reported as "partly cloudy" days be considered as clear, we have 27 additional days, making a total of 146, or 68.2 per cent of the growing period. This is practically identical with the approximation of 145 days arrived at by a similar process for

the growing season of 1920. It must be remembered, however, that this total was not available for the uninterrupted growth of the crop, as in other years, since the killing of shoots on March 29 and April 11–12 retarded the development of foliage and the production of bloom by approximately three weeks. There was a twofold effect upon the plants; their food reserves were reduced in direct proportion to the amount of growth at the time of the freeze, and there was also a reduction in the period of photosynthetic activity within which the development of foliage, the production of fruit, and the accumulation of stored reserves for next season's growth had to occur. This reduction of the effective photosynthetic period, considered alone, would be expected to produce a crop characterized by lower carbohydrate content than that of crops of other years. Other conditions were operating to mimimize this reduction, however, in that the maximum and mean temperatures were consistently higher than average throughout the growing season except in August, and the vines bore crops of fruit ranging from 30 to 80 per cent of the normal as result of the frosts. The fruit was adjudged in the field to be almost or quite up to the average dessert quality. Ripening was somewhat hastened, early and late maturing varieties ripening together. Picking was begun September 16 and completed on the 29th, several days earlier than usual. November was warm and rainy, the first killing frost occurring on the 6th, and severe cold and snow did not set in until the middle of December.

Climatic Record for 1922

The deficiency of soil water resulting from the drought of 1921 was not made up in the early months of 1922, as the rainfall for the first five months totaled only 17.09 inches as compared with a normal of 18.81 inches for the period. The mean temperatures from January on through June were consistently 2.8° to 5° above normal, but periods of cool weather at the middle and end of March and until after the middle of April retarded plant growth and prevented the forcing of buds, and when a temperature of 30° occurred April 23 and 24 it did no damage in the vineyard (fig. 10). June and July were excessively wet, with 14.98 inches of rainfall, or one-third the total for the year. The rains of June were distributed mainly as showers over 15 days of the month. July had approximately the normal number of clear days in spite of more than twice the normal precipitation. August, like June, had a large number of cloudy, showery days and consequently less than normal sunshine, but the August rainfall was 0.5 inch below normal (fig. 11). The mean temperature was subnormal by

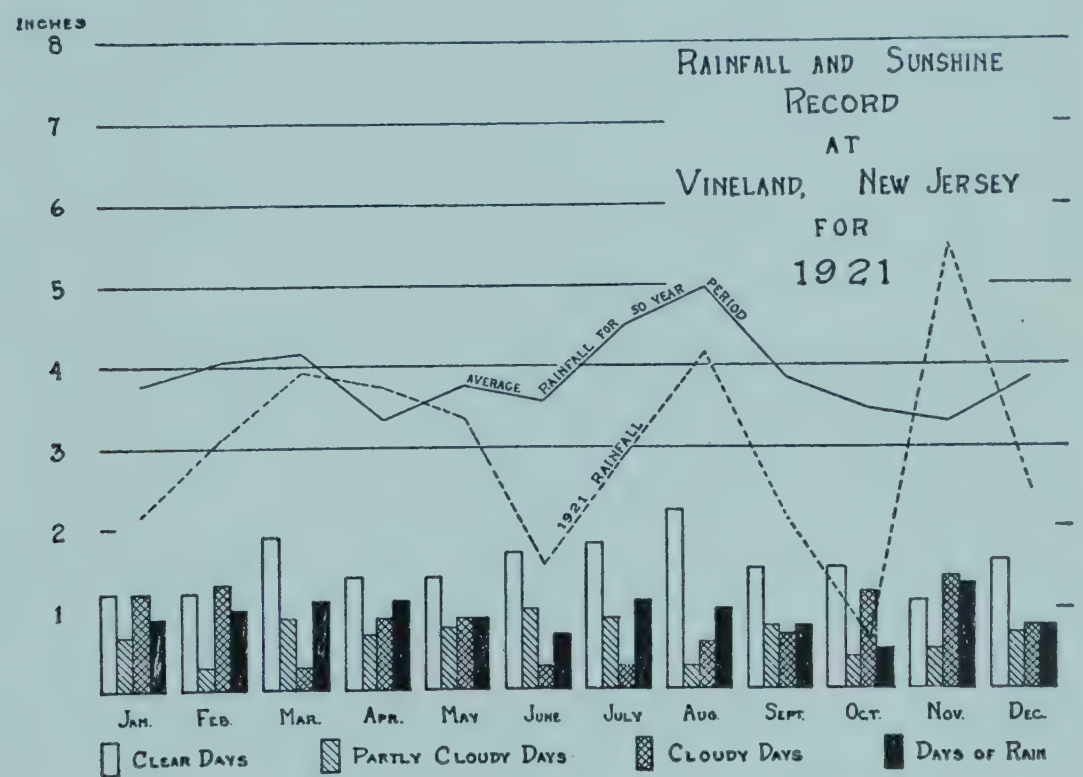

Fig. 9.—Rainfall and sunshine at Vineland, N. J., in 1921, with 50-year average for comparison

about 1° through July and August, rising again in September and remaining above average for the remainder of the year. September had more than the average amount of sunshine and only about two-thirds of the normal precipitation (which occurred on the 1st and 24th), and clear, hot weather continued through the middle of October. The crop matured perfectly and a little earlier than usual, picking having been begun September 14 and completed October 2. The crop was somewhat smaller in quantity than in other years, possibly about 85 per cent of normal, but was unusually uniform in that practically all varieties bore about the same amounts. The vines made exceptionally vigorous growth and the unusually heavy foliage was well retained until after the close of picking.

In point of sunshine received during the growing season—March to September, inclusive—the year was the least favorable of the entire period. It had 114 clear days, with 50 recorded as "partly cloudy." Applying the arbitrary method of valuation of partly cloudy days previously used, the whole period had the equivalent of 139 clear days, or 64.95 per cent, a smaller total than for any other year of the period. It is also worthy of note that that shortage of sunshine occurred in June and August, June having 11 clear and 10 partly cloudy days and August 12 clear and 10 partly cloudy days. The total of 139 days of sunshine probably permitted materially more photosynthetic activity than the 146 days of a like period in 1921 when the effect of frost in that year is considered, but it must also be remembered that the vines were bearing a considerably larger load of fruit in 1922 than they had in 1921, which would operate to reduce the carbohydrate content of the fruit.

The first killing frost of the autumn occurred October 21, about two weeks earlier than usual. October had a little more than one-third, November only one-fourth, the usual rainfall, and the mean temperatures were 3° to 4° higher than the average. December was very slightly above normal in rainfall and temperature with only 2.8 inches of snow.

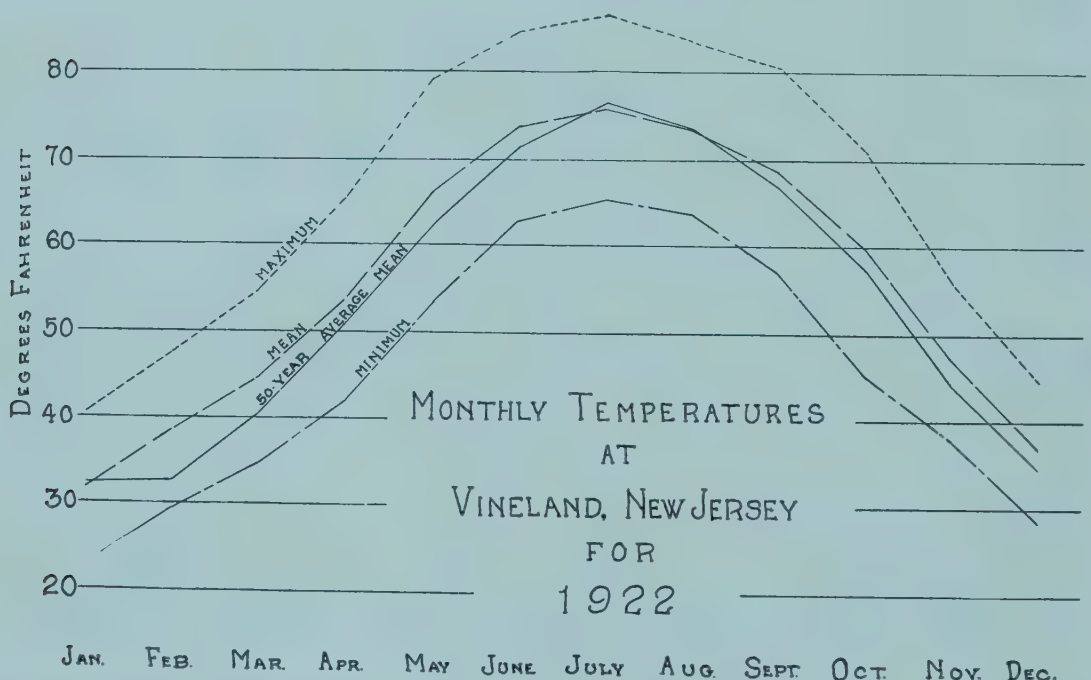

FIG. 10.—Maximum, minimum, and mean monthly temperatures at Vineland, N. J., in 1922, with 50-year average for comparison

CLIMATIC RECORD FOR 1923

The winter of 1923 showed no unusual features aside from a prolonged cold period in the last half of February and a cold wave at the beginning of April. The resumption of growth was later than usual and was retarded considerably by cool weather in the first three weeks of May, which was very dry. June was the hottest month of the year, with high maximum temperatures, but the mean temperature was almost constant for June, July, and August, considerably exceeding the 50-year average in June, dropping a little below it in July, and rising in August to remain slightly above normal through November (fig. 12).

The distribution of precipitation during the first 11 months of 1923 departed widely from the 50-year average, as the precipitation was excessive in the winter months, then decidedly below normal in the period May to August, inclusive, then slightly above normal in September and October (fig. 13). The deficiency in precipitation up to December 1 was only 3.96 inches, but the fact that the four months May to August inclusive, received only 7.88 inches instead of the normal 16.58 made the period of development of the crop an unusually dry one. This condition was accentuated by the fact that much of the rainfall of May, June, and July was in the form of light showers of a few hundredths of an inch each, the only rains of 0.5 inch or more during the period being on June 29, July 3, and July 11. The precipitation of August was mainly in the form of three rains exceeding 0.6 inch each on the 5th, 20th, and 29th. The September rainfall, while exceeding the average in amount, occurred mainly in two rains of 1.58 and 2.20 inches on the 8th and 23d. October was rainless until after the completion of grape harvest on the 11th. Consequently the crop, which was the largest since 1918, ripened in a period of warm, bright, rainless weather and was considered at the time of picking to be the best crop of the period of observation.

The total number of clear days in the period March to September, inclusive, was 120, while 50 days were reported as partly cloudy. Employing the method of evaluating partly cloudy days previously used, the 214 days of the growing period had 150 days of sunshine, or 70.09 per cent. This is a larger percentage of sunshine than had been received by the crop during an equal period since 1919, but was somewhat below that of 1918 and 1919. It was unequally distributed over the period; the first four months had little cloudy weather and consequently received a total of 89 days of sunshine, or 22 per month; July, August, and September had unusually large percentages of partly cloudy days, only 12, 16, and 15 days, respectively, being reported as clear. While the total number of days of sunshine for the three months by our method of evaluation is 61, or 20 per month, the very considerable extent to which doubtfully valuable partly cloudy days enter into this total makes it certain that these months were less favorable for photosynthetic activity than the four months which preceded them. This was indicated by the late ripening

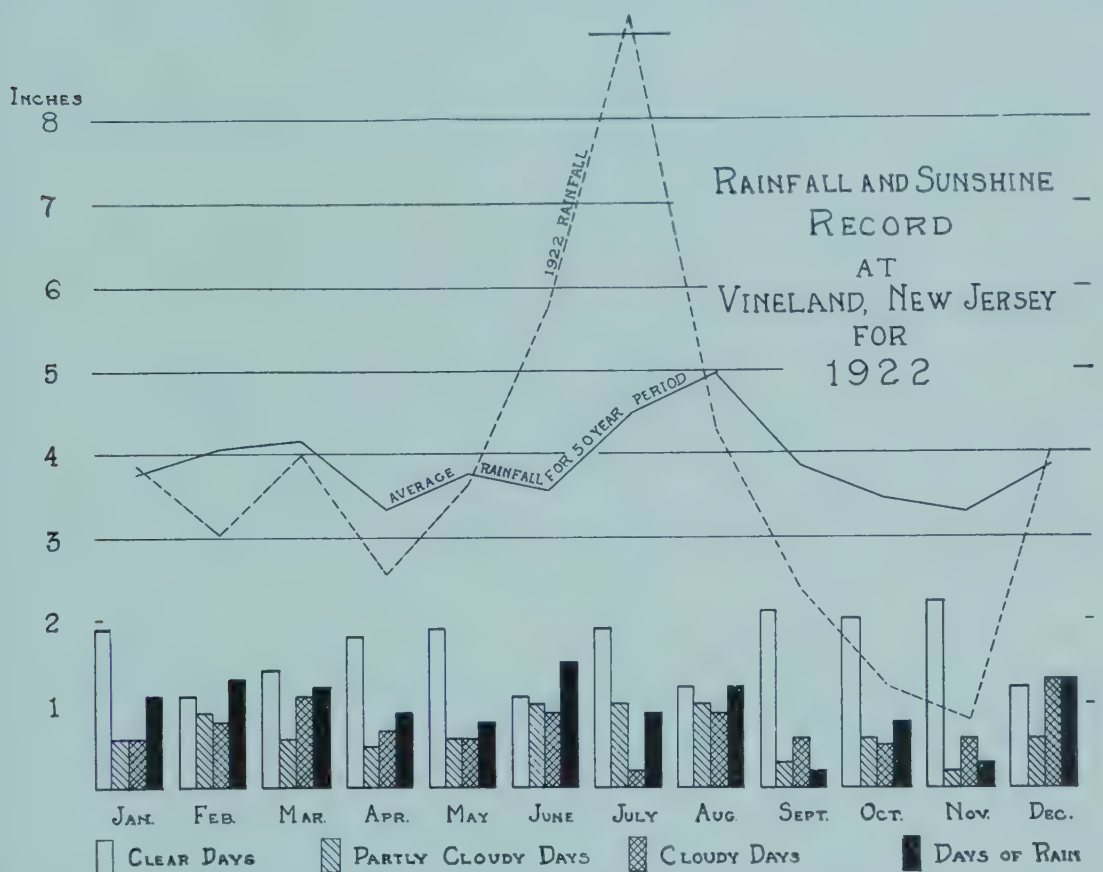

FIG. 11.—Rainfall and sunshine at Vineland, N. J., in 1922, with 50-year average for comparison. (All days on which a trace, 0.1 inch or less, was recorded are included)

of nearly all varieties, as picking was begun September 20 and extended through October 11, several days later than usual. In some degree the lateness of ripening added to the amount of sunshine received, as the whole period, September 20 to October 11, was one of clear warm weather, broken by rain only on September 23. The various varieties thus received 4 to 10 days of uninterrupted sunshine after the close of the period which has been considered for purposes of comparison. That there was very little if any actual shortage of sunshine as compared with 1918 or 1919 is indicated by the fact that the crop, which was the largest harvested during the five-year period, ripened normally, full characteristic color being developed in those varieties which do not color well in an unfavorable season. With practically no exceptions, the fruit of all varieties was regarded at picking time as of dessert quality fully equal to that of any previous year.

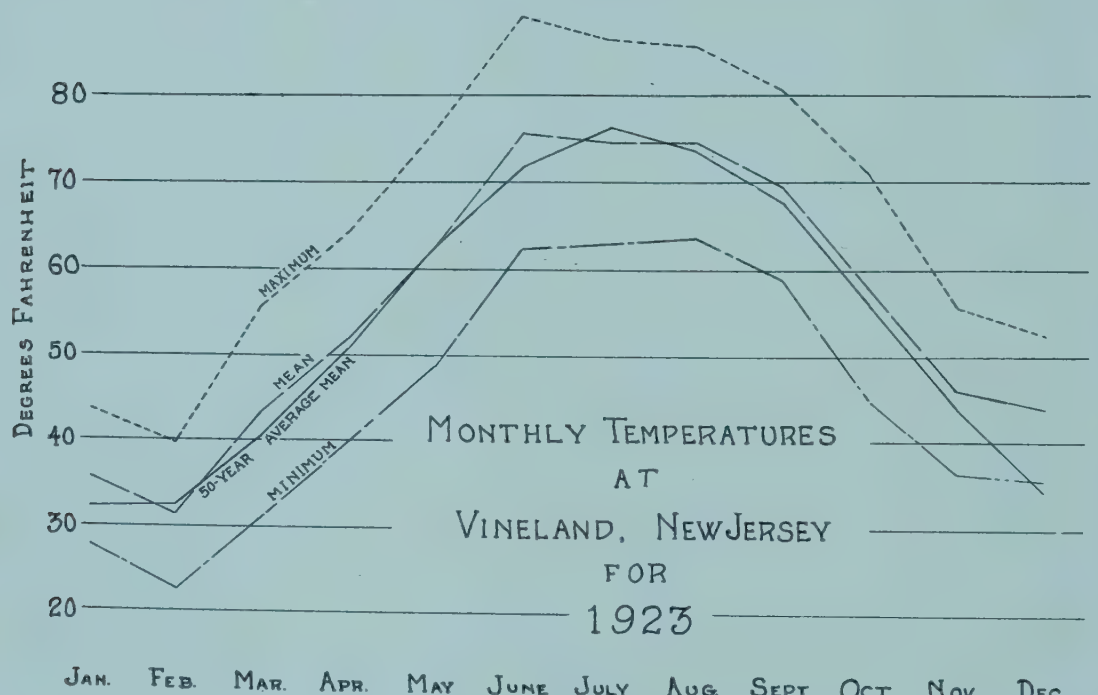

FIG 12.—Maximum, minimum, and mean monthly temperatures at Vineland, N. J., in 1923, with 50-year average for comparison

CLIMATIC CONDITIONS AS RELATED TO VARIATIONS IN CHEMICAL COMPOSITION OF THE CROP

The seasonal conditions prevailing during the period covered by the studies of the crop have been described in some detail. The analytical results have been presented and the character and extent of the fluctuations in content of sugar, acid, and astringent constituents have been discussed. It remains to bring the climatological data and the analytical results together and to determine whether variations in the conditions under which the crop is produced exert discoverable and consistent effects upon the chemical composition of the fruit. If consistent mass effects appear under certain seasonal conditions, i. e., if a majority of a large number of varieties behave as one with respect to the chemical character of the crop, after individual variations have been eliminated by using composite samples made up of the entire crops of a considerable number of plants of each variety, one may feel warranted in the conclusion that seasonal factors are responsible for such mass effects.

The writer knows of no very extensive or exact studies upon this point to be found in the literature. Munson (*49*) publishes sugar and acid determinations, made with Oeschles' scale and Twitchell's acidometer, upon juices of 89 varieties grown at Denison, Tex., in 1906 and 1907. He remarks that the season of 1906 was excessively rainy and cool, and that the readings for sugar are all 5 to 20 points (Oeschle's scale) lower and the acid readings 1 to 4 points (Twichell's instrument) higher than in 1907, which was a much warmer and drier season (*49, pp. 123–125*), saying further that

> it is true that the degrees of sweet and acid vary with season, soil, condition and age of vines, and with change of weather, but varieties generally maintain their relative positions throughout the changes, with some unimportant exceptions.

Alwood (*2*, *3*) notes in connection with his studies of the development of sugar and acid in grapes during ripening, which were carried on in the Lake Erie district in 1908, 1909, and 1910, that the crop of 1908 was one of very exceptional quality, while that of 1909 was remarkably heavy, but that cold, rainy weather extended over the whole ripening period, some late varieties being partially defoliated and failing to ripen as a result. The crop of 1910 was reduced by May frost to about 40 to 60 per cent of normal. Alwood remarks that the sugar content of several varieties was 1 to 4 per cent lower, and the acidity was higher, in 1909 than in 1908, but does not present data as to the seasonal conditions prevailing during the other years of the study. The work of Kelhofer (*38*) and of Müller-Thurgau (*48*) on this point has already been mentioned (p. 1153).

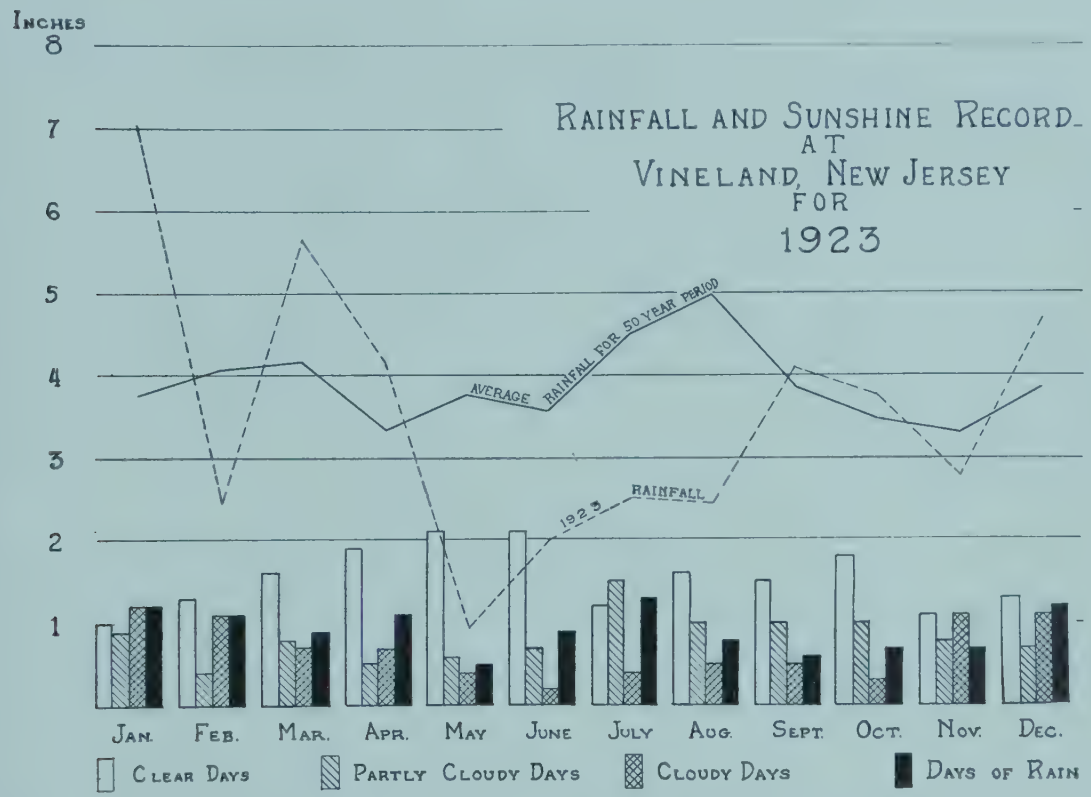

FIG 13.—Rainfall and sunshine at Vineland, N. J., in 1923, with 50-year average for comparison. (All days on which a trace, 0.1 inch or less, was recorded are included)

The five crop years in which analytical data was secured have been roughly compared with one another in the discussion of the climatological data in a preceding section. Such a comparison involves a consideration of the varying factors—amount and distribution of rainfall and humidity, temperature, and sunlight—which enter into the makeup of a crop season, to the end that each may be evaluated with respect to its effect upon the plant, before any definite picture of the sum of all these effects can be obtained. Hedrick (*29*, *30*) has summarized climate, as it affects grape growing, in these six essentials: Length of season, seasonal sum of heat, amount of humidity in summer, dates of spring and autumn frosts, winter temperature, and air currents. While this basis holds good when distinct areas differing materially one from another in some of these factors are being compared, it does not apply to the present case. The climatic conditions at Vineland are such as to permit annual bearing of crops of normal size by a large number of varieties of early, medium, and late grapes. None of the elements of climate as enumerated by Hedrick is sufficiently variable to become a limiting factor upon fruitfulness. The analysis necessary here is upon a finer scale, since the variations encountered are such as merely to modify the crop in quality and chemical composition without markedly affecting the growth or vigor of the plants. As has been pointed out by Livingston and McLean (*44*), the growth of the plant automatically integrates all the fluctuating elements of the environmental complex, and the growth of a selected plant has been used as a means of measuring climatic conditions in different parts of an area, as in the work of McLean (*45*) and Hildebrandt (*31*). In the work of these writers the climatic

efficiency of various selected stations for growth of soy beans (variety Peking) was determined by growing the plants for four weeks from seed under controlled conditions and determining stem height, leaf area, and dry weight of tops. Hildebrandt has shown that there is no very close agreement between the instrumental measurements of the climatic data and the actual growth of the plants, the growth in some cases being greater, in others less, than expectation based upon climatic data. This study approaches the same general problem from a different angle, in that the plant employed is a long-lived perennial, and the climatic conditions are those of a single locality and did not vary widely enough to affect the annual growth or fruitfulness of the plants but only enough to alter the chemical composition of the fruit. It seems logical to assume that the automatic integration of the environmental complex by a perennial plant will be reflected in the chemical composition of the ripe fruit as truly as it is in the elongation and increase in dry weight of an annual.

If this assumption be true, it remains to be determined whether it is possible, by examination of the ordinary weather records of temperature, humidity, sunlight, and rainfall, to reach a dependable conclusion as to the nature of the effects which a particular set of seasonal conditions will have upon the composition of the crop. These factors are so intimately interrelated that even under the most complete control of experimental conditions thus far attained, it is difficult or impossible to modify the amount of sunlight received by a plant without modifying temperature and humidity. With plants growing in the open the mass effect of this interplay of factors is observed, but an attempt to analyze the result or determine the relative importance of the rôles played by the individual factors is usually considered unpromising. There is a possibility, however, that the interrelationship of these factors is of such a nature that they operate together to favor or depress photosynthetic activity in the plant. Thus excessive and long-continued rainfall is associated with decreased sunlight and lowered temperature, all of which operate together to depress the rate of photosynthesis, while drought implies maximum sunlight and high temperatures, which increase the rate so long as the plant has available an adequate water supply. Of course extreme modification of any factor may result in disturbance of metabolism and death, but within the limits of normal plant behavior seasonal changes consist in the modification of several or all the factors named in mass fashion, in a direction favorable or unfavorable to the plant. If this interlocking of climatic factors is sufficiently intimate and constant within the range of conditions in which normal growth is possible, it should be possible to find among the environmental factors some one which can readily be accurately measured and which will give a dependable indication of the direction in which all the factors have operated upon the plant during the season.

Under the conditions prevailing at Vineland the sunlight received during the growing period is such a factor. It is the factor which varies most widely from year to year. The nearness of the locality to the ocean renders it subject to fogs and periods of cloudiness and rain which extend over a very limited area and which cut off sunlight with much less accompanying depression of temperature than occurs during general rain over a wide area. Nearness to the ocean is also responsible for the absence of large differences in relative humidity and in temperature when the records of a number of seasons are compared. The equalizing effect of the ocean upon these factors throws the variable factors, rainfall and sunlight, into stronger relief than might be the case far inland.

Under these conditions there is a quite constant agreement between the sunlight received by the vines during the season and the composition of the crop. This of course does not mean that sunlight is the sole significant factor in determining the chemical composition of the crop but merely that in the locality of Vineland it is the dominant factor, other climatic factors playing subordinate parts and exerting their effects in the same direction. Whether there is such a correlation in regions remote from large bodies of water can be determined only by future work.

It is scarcely possible that the variations in amount and distribution of rainfall during the period exerted any direct effect upon the composition of the crop. The period as a whole was one of subnormal rainfall, the accumulated shortage for the years 1917 to 1923 inclusive being 15.76 inches, although one year (1919), had an excess of 12.61 inches. At no time during the period did the vines display evidences of distress from a shortage of water, although other crops suffered severely from drought in 1921, when there was a deficiency of 10.07 inches. As the vegetative

vigor and general condition of the vines in that year, with a total rainfall of 35.57 inches, compared favorably with that of 1919, with a total rainfall of 58.25 inches, as well as with that of other years of more nearly normal precipitation, the variations in rainfall can not have played a determining rôle in chemically modifying the crop.

Records of relative humidity were not kept at the Vineland station during the work. Detailed records are kept at Atlantic City and Philadelphia. Examination of these records indicates that the departures from normal humidity year after year are so inconsiderable as to make this factor of no significance, and the location of Vineland makes it very probable that the same conditions prevail there. The Vineland temperature records show very slight departures from the 50-year mean, which is no doubt due to nearness to the seacoast. These departures are too small to be significant when considered alone.

The climatic factor which stands out as varying most widely from year to year is the amount of sunlight received by the plants during the growing period. Location subjects the Vineland territory to occasional periods of overcast, cloudy weather at any time during the growing season and to frequent showers and fog in the late summer. The number of clear days in the period March 1–September 30, inclusive, varied as follows: 1919, 134; 1920, 110; 1921, 119; 1922, 114; 1923, 120. As pointed out in the discussion of climatic conditions, the number of clear days alone does not give an accurate idea as to the seasonal sunshine received, as partly cloudy days also contribute some sunshine. The number of days reported as partly cloudy are: 1919, 42; 1920, 70; 1921, 54; 1922, 50; 1923, 60. In the system of recording used by the Weather Bureau branch stations, days having four-tenths to seven-tenths of the hours of possible sunshine prevented by clouds are classed as partly cloudy. It has been assumed that in the aggregate the partly cloudy days furnish 50 per cent of the possible sunshine. It is probable that this approximates the actual facts as closely as is possible in the absence of an accurate detailed record.[12] The taking of 50 per cent as an average very probably somewhat underestimates the actual amount of sunshine received, but does not affect the results for purposes of comparison. Proceeding upon this assumption and adding to the clear days for each year one-half the number of partly cloudy days, we have for 1919, 155 days; for 1920, 145 days; for 1921, 146 days; for 1922, 139 days; and for 1923, 150 days. These figures present as close an approximation to the amount of sunlight in each of the years as can be arrived at from the data in hand. Ranked in the order of amount of sunlight, 1919 stands first, 1923 second, 1920 third, and 1922 fourth. 1921 must be separately considered, since the young shoots of most varieties were killed by frost, which retarded development for two or three weeks and reduced the size of the crop in that year. Consequently the amount of sunlight received during the season can not be used as a basis for comparing the crop with those of other years, as an unknown portion of the time, varying with variety, was required for recovery from the injury and the crop produced was subnormal in amount.

As has been pointed out in the discussion of the analytical data, 1923 had a slightly larger number of varieties attaining maximum sugar content than 1919, although 1919 had five days more sunlight in the period chosen for comparison. It was stated in the discussion of climatic conditions for 1923 that the year was one of late ripening, the harvest period extending through October 11, the fruit of the majority of the varieties remaining on the vines a week to 10 days longer than usual. As the period September 20 to October 11, inclusive, was one of high temperature and uninterrupted sunshine, conditions for photosynthetic activity were optimum, and the crop stored considerable sugar after the close of September. It is probable that by reason of this fact that this crop received fully as much sunlight as did that of 1919. The two years are characterized by the large number of varieties having high sugar, 1919 having 15 with maximum and 12 with next-to-maximum quantities, while 1923 had 18 with maximum and 17 with next-to-maximum contents. That years of high sunshine are also years of generally low acidity is also clear, 1919 having the maximum in 20 varieties and next-to-maximum in 9. Astringency determinations were made only for the years 1921–1923, inclusive,

[12] It is probable that there is considerable variation in the use of the term "partly cloudy" by different observers. For example, the record for March, 1921, at Atlantic City, states that the 8th, 12th, 21st, 22d and 27th had 92, 97, 89, 91, and 98 per cent of possible sunshine, yet it classes them as partly cloudy, while March 14, 1923, is classed as clear, although it is recorded as having 69 per cent of possible sunshine. July 9, 1923, is recorded as partly cloudy, yet as having 100 per cent possible sunshine. (Monthly Meteorological Summary, Atlantic City, N. J., Form 1030, for dates given.)

but the year 1923 was remarkable for the fact that 42 varieties out of 49 showed minimum total astringency for the three-year period in that year. This was due to the fact that both true tannins and astringent nontannins were low, 20 varieties having minimum tannin while 44 had minimum nontannin in 1923. From the results one might conclude that in this year high sugar content was associated with low acidity and low total astringency, and that the latter was due to a low content both of true tannin and astringent nontannins.

The year 1920 had a total of 145 days sunlight as the effective period for photosynthetic activity. In sugar content the crop ran low, only 4 varieties showing the maximum, while 13 had minimum and 14 next-to-minimum figures. In acid content there were 13 with maximum and 8 with next-to-maximum content. The general tendency is to run medium to low in sugar and high to medium in acid, minimum acid and maximum sugar being equally rare. No astringency determinations were made on the fresh juice.

The year 1922 was the least favorable for photosynthetic activity of the four comparable years, having only 139 days of sunshine in the crop period. The sugar content in the crop as a whole was lower than in 1923, 17 varieties having minimum and 10 next-to-minimum content, while only two had maxima. Associated with low sugar was an outstanding high acidity, 19 varieties having maximum and 17 next-to-maximum acid content. With high acid readings there was associated distinctly high astringency, 35 having the maximum for the three-year period. Thirty-three had maximum true tannins, while 27 had maximum astringent nontannins. Here low sugar content, high acidity, and high total astringency resulting from high tannin and high astringent nontannins are found associated. This fact, coupled with the fact that the fruit ripened somewhat earlier than usual, led to delay in picking some varieties until they were slightly overripe. The flavor of the fruit was so affected by the altered composition that it was difficult to convince one's self that it was fully ripe even when it showed all the physical signs of ripeness.

This review of the data for the four years of normal crop conditions indicates that there is a considerable degree of correlation between the fluctuations in certain chemical constituents of the grape. High sugar content is associated with low or less-than-medium acidity and total astringency; low sugar content is associated with high acidity and high total astringency. While there are individual exceptions, there is a clearly marked mass tendency, the majority of varieties behaving as one throughout the period. It is clear that this mass behavior is a response to the seasonal conditions of the years concerned. The year 1921 has thus far been excluded from consideration by reason of the abnormal conditions of that year, namely, a spring freeze with resulting shortening of the growing period and reduction of the crop, which precludes consideration of the data on the same basis as those of other years. In view of the correlation between sugar, acid, and astringency found to exist in the other years, it becomes a matter of considerable interest to know whether this correlation continues to hold or breaks down in an "off-year" of abnormal conditions and reduced crop. The data for 1921 will therefore be reviewed somewhat in detail for the light it may throw upon this point.

The graphic summary on sugar and acid content, Figure 1, can not be employed for this purpose, for the reason that it summarizes results which must be segregated in order to be properly interpreted.

As stated in the discussion of seasonal conditions for 1921 (p. 1164), 13 varieties suffered no injury from the frost. Ten of these, namely, Agawam, Cynthiana, Franklin, Herbemont, Lenoir, Missouri Riesling, Noah, Norton, Vergennes, and Wilder, bore crops of full normal size. The analytical data for these is entirely comparable with that for the other years, and may be considered on the basis of the sunlight received, which exceeded by one day that of 1920. Reviewing the data for these 10 varieties separately, 1 has maximum and 1 next-to-maximum sugar, 4 stand in third place, 3 in fourth place, and 1 has the minimum. In acid 1 has maximum, 1 next-to-maximum, 3 in third and 3 in fourth place, while 2 are minimum. The majority of the varieties are consequently midway between extremes, thus recalling the situation found in 1920. In astringent constituents, 4 have maximum total astringency, 1 has the minimum, and 5 are intermediate. In true tannins 2 have the maximum, 8 the minimum, while 6 have maximum nontannins, and 4 next-to-maximum. The general situation with these 10 varieties is therefore one of medium sugar and medium acid content with

medium to high total astringency, high astringency being due in the majority of cases to a high content of astringent nontannins.

The remaining varieties suffered more or less severely from the freeze, they losing two to three weeks of the effective period for photosynthetic activity and their crops were reduced to 30 to 85 per cent of the normal. It is of interest to ascertain to what extent the crop produced under the abnormal conditions tends to show the same correlations in composition as are found in other years. Summarizing the data, we find that 9 varieties had maximum, 7 next-to-maximum sugar content, 4 stood in third place, 9 in fourth place, and 10 had the minimum. In acid content there were no maximum, 7 next-to-maximum, 17 in third place, 10 next-to-minimum, and 5 minimum. In total astringency 9 had the maximum, 3 the minimum, and 25 were intermediate. As regards true tannins, 6 had the maximum, 16 the minimum, and 15 were intermediate, while 15 had maximum nontannins and 21 an intermediate amount, there being none with the minimum. The majority of these varieties occupy an intermediate position in their sugar, acid, and total astringency, but there are some maximum and minimum results upon sugar. An examination of these in detail will constitute a rather crucial test of the persistence of the correlation found elsewhere, since the varieties having maximum sugar were those having greatest reduction in the crop (40 per cent or less) while those showing minimum sugar had 60 to 85 per cent of a normal crop. (See Table III.) Expressed in another way, plants having comparable amounts of foliage were storing sugar in crops of very unequal size; the plant with a very small crop brought its fruit to a high sugar content in the same time and under the same conditions that permitted the plant with a crop of approximately normal size to store only a minimum.

Of the 9 varieties attaining maximum sugar content in 1921, 3 had minimum acid, 2 next-to-mimimum, and 4 stood in third place, there being no maximum or next-to-maximum cases. Two had maximum and 7 next-to-maximum astringency. For the varieties having minimum sugar content, 5 had next-to-maximum acidity, 3 stood in the third place, and 1 in fourth, while 6 had maximum and 3 next-to-maximum total astringency. Thus, nearly all the cases of extremes in sugar, acid, and total astringency are related, high sugar being accompanied by low acid and astringency and low sugar by the opposite condition. The remainder of the crop was made up of individuals characterized by the absence of extremes in any particular, sugar, acid, and astringent constituents alike being intermediate in amount between the high and low figures occurring in other years.

Summarizing, two outstanding conclusions are to be deduced from the results of this study. First, the amount of sunshine received during the period March 1 to September 30, inclusive, is, for the five years of this study, a dependable index of the sugar content of the crop of grapes produced by a large number of varieties each of which was bearing annual crops of average or normal size. Under the conditions of the experiment, the amount of sunshine received during the growing season is the only climatic factor which varied widely from year to year, hence it determined the amount of photosynthetic product accumulated in the crop. While there are individual exceptions, the majority of varieties behave quite uniformly as one throughout the experimental period.

Second, there is a consistent and fairly high degree of correlation between sugar, acid, and total astringent content, extending through the experimental period. The nature of this relationship is such that a high content of sugar is found associated with low or medium content of acid and of astringent constituents, a low content of sugar is associated with high acidity and astringency, while an absence of extremely high or low sugar content is accompanied by an absence of extremes in acid and astringency. While there are some exceptions, this general statement holds not only for four years of normal crops but also for a year in which frost injury materially reduced the crop. In every year there is an evident mass tendency to behave in the manner stated.

The explanation of this situation would appear to be that the content of each of these constituents present in the grape at maturity is conditioned by the amount of sunlight received by the plant during the formation of the crop. Conditions which favor a maximum accumulation of the products of photosynthesis in the fruit also favor the reduction of the content of acid and astringent constituents to a low or minimum point. Conditions which minimize photosynthetic activity depress the accumulation of sugar in the fruit to a minimum and at the same time depress the reduction of

acidity and astringency, leaving these constituents at a high level. Intermediate conditions result in the production of a crop characterized by the general absence of extremes in the amount of any of these constituents. There are occasional exceptions to each of these statements to be found in the data, but there is a clear general tendency to behave in the manner indicated.

There is no immediate or rigid relationship between acid content and total astringency as regards their behavior under a given condition; both react in the same direction but in varying degree. The same statement holds for the gelatin-precipitable and nontannin constituents grouped together as total astringent substances. The amounts of tannin and nontannins present fluctuate rather widely from year to year, and these fluctuations are to a considerable degree independent, and in consequence there are a number of cases in which total astringency content does not fulfill expectation for the reason that the two constituents did not behave in the same way. A general tendency is clear, however, and it is possible that the accumulation of more data, together with the extension of our knowledge as to the real nature of the substances at present grouped under the designation astringent nontannins, would furnish an explanation of the inconsistencies in behavior here reported.

It would seem in the light of the results given here that the climatic conditions during the growing period of the grape crop, or more specifically the amount of sunshine received, when other conditions are constant, bear such a relation to the chemical character of the crop that the general chemical character of the crop may be predicted from a knowledge of the effective period of photosynthetic activity.

SUMMARY

Chemical analyses of the juices of a collection of 66 varieties of grapes grown together under controlled conditions at Vineland, N. J., were made annually during the five-year period 1919 to 1923, inclusive. The determinations made included total solids, free reducing sugars, total sugars after inversion, titratable acidity, total astringency, and nontannin astringency.

The range of variation in composition of the juice of any variety grown under constant conditions in one locality through a series of years is much narrower than the variation encountered when a large number of samples of a given variety grown over a wide area and under a variety of cultural conditions are compared. Differences of soil and cultural conditions appear to affect varietal composition more profoundly than do variations in environmental conditions encountered over a series of years in one location.

Cane sugar is of widespread but very irregular occurrence in the varieties of grapes here studied, having been found at least as a trace in all but one variety. Its presence is in some degree indicative of immaturity of the fruit, but it is often found in fully mature or overripe grapes. Its presence can not be definitely correlated with any other factor such as temperature, insolation, water supply, or load of fruit borne by the vine.

There is a very definite and clearly marked effect of climatic conditions during the growing season upon the total sugar content, total astringency, and titratable acidity of the fruit produced during that year. This effect is a mass effect, all the varieties responding to a given set of climatic conditions in the same manner.

Of the various climatic factors entering into the composition of the seasonal conditions at Vineland, the amount of sunshine received during the period March to September, inclusive, is subject to the greatest annual variations and is the dominant factor in determining the chemical character of the crop.

Under the conditions prevailing at Vineland, the content of sugar, acid, and astringent substances present in the juices of fully ripened grapes from normally loaded vines seems to be determined very largely if not altogether by the number of days of sunshine occurring in the period of 214 days between March 1 and September 30.

In vines bearing annual crops of comparable size, the year of maximum sunshine during the period March to September, inclusive, was the year of maximum or next-to-maximum sugar content of juice in a majority of all varieties. The year of minimum sunshine for the period March to September, inclusive, was the year of minimum or next-to-minimum sugar content for a majority of all varieties in which the vines bore normal crops. Years having amounts of sunshine intermediate between these extremes during the growing period have amounts of sugar intermediate between the extremes and proportional to the amount of sunshine received.

The year of maximum sunshine during the growing period was the year of minimum acid content and minimum total astringency content in a majority of varieties, while the year of minimum sunshine during the growing season was the year of maximum titratable acidity and total astringent content in a very large number of varieties. Years intermediate between the extremes in amount of sunshine received are intermediate in the amounts of acid and astringent materials present, and these amounts are inversely proportional to the amount of sunshine for the growing season.

Conditions which permit a maximum accumulation of sugar in the fruit also favor the reduction of the titratable acidity and astringent content to a low or minimum value. Conditions which minimize photosynthetic activity and accumulation of sugar depress the reduction of titratable acidity and astringent content of the fruit.

Titratable acidity and total astringency of a fruit juice are affected in the same direction, but independently and in varying degree, by the conditions prevailing during the maturing and ripening of the fruit.

Under the environmental conditions in which this work was carried out, the amount of sunshine received during the period of growth is the dominant factor in determining the chemical character of the fruit of a large number of grape varieties of widely differing parentage and character of fruit. A knowledge of this factor, when the climatic factors vary within a relatively narrow range as at Vineland, may enable one to forecast the general chemical character of the crop to be produced.

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NATURAL REPRODUCTION AFTER FOREST FIRES IN NORTHERN IDAHO[1]

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INTRODUCTION

This study of natural reproduction following some of the largest and most destructive forest fires in northern Idaho was made, in 1923, with the object of discovering in what way and to what relative extent the several species of conifers reseed the ground, and to learn what factors, such as intensity of the fire, differences in site or stand conditions, or seed production of the forest, affect natural restocking.

In the fall of 1923 studies were made on large burns on the Coeur d'Alene National Forest—on areas burned only once, which was in 1910, and on areas burned twice at different intervals by fires which occurred in 1870, 1889, 1910, and 1919.

FIRE HISTORY

BURN OF 1870

The earliest large burn studied occurred about 1870. This burn killed approximately 3,200 acres of mature timber on Trail Creek and its tributary, Bear Creek, a mile or so to the west of Magee Ranger Station. (See fig. 1.) The numerous groups of seed trees which still exist on the higher points, as well as the comparatively small area burned, strongly indicate that conditions were not so arid nor the fire so intense and destructive as in the burns of 1910 and 1919. The Trail Creek area reseeded quite generally after the 1870 burn, but the stand that followed was almost completely killed in 1910. Here opportunity was afforded for a study of reproduction after fire had destroyed a young 40-year stand.

BURNS OF 1889 AND 1910

A later burn of greater consequence, that of 1889 on the Deep Creek area, covered the upper south and west slopes along the Idaho-Montana divide. This was part of a very general fire which originated near Pend Oreille Lake and swept large areas in the Clark Fork Valley to the north. These areas reproduced well but burned again in 1910, leaving very little inflammable material, so little in fact as to prevent the 1919 fire from crossing over into Montana. The coincidence here of the 1889 and 1919 burns permitted the study of natural reproduction following the destruction of a 30-year stand.

The large and very destructive fire of 1910 occurred about August 20. It began in the Magee district and swept in a path 7 to 9 miles wide and 18 miles long to the Idaho-Montana divide. The destruction was quite general and complete. Only here and there on bottoms, lower north slopes, high points, or on very rocky sites did groups of trees survive. The most clear-cut cases of single 1910 burns were studied on Alder Creek.

BURN OF 1919

The large fire of 1919 made a fairly complete clean-up of the dead material left by the 1910 burn, covering high and low points alike. It stopped when it reached the upper south slopes of the Idaho-Montana divide, laid bare by the two earlier fires of 1889 and 1910. Opportunity was afforded here for a study of natural reproduction following a very large and intense double burn, on a thousand acres of which there remained not one live tree. In two other sections with large open north and south aspects only a few isolated groups of green trees survived both fires.

CHARACTERISTICS OF BURNED AREAS

The study as a whole gave evidence of certain outstanding typical conditions which appear and reappear on different parts of large burned areas and which are due to similarity of slope, aspect, and elevation. That is, where the topography is similar the results from burns are similar, provided

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the stand or forest is uniform. This principle, of necessity, is of first importance in the application of the data obtained by this study. On this basis, the results of single or repeated burns are treated separately according to the following topographic subdivisions:

1. Lower north and east aspects.
2. Middle north and east aspects.
3. Upper north and east aspects.
4. Lower south and west aspects.
5. Middle south and west aspects.
6. Upper south and west aspects.
7. Bottoms.
8. Draws.
9. Benches.
10. Tops.

Further subdivisions of each of the above locations may be made according to minor local variations of each.

DEEP CREEK AREA: BURNS OF 1910, 1919, AND 1889

The Deep Creek area was selected because the large and severe fires of 1910 and 1919 ran rampant there in virgin western white pine timber, and because there had been neither grazing nor lumbering either before or after the fires (pl. 1, B).

Descriptions and records of the forest composition previous to 1910 do not exist. It is evident from what now remains that the entire watershed contained magnificent stands from 200 to 300 years old of western white pine (*Pinus monticola*), western larch (*Larix occidentalis*), Douglas fir (*Pseudotsuga taxifolia*), western hemlock (*Tsuga heterophylla*), lodgepole pine (*Pinus contorta*), Engelmann spruce (*Picea engelmanni*), lowland fir (*Abies grandis*), and alpine fir (*A. lasiocarpa*). The white pine occurred on all aspects up to 4,500 feet, giving way to pure Douglas fir only on the high south slopes. Engelmann spruce was confined to the stream bottoms and lower north slopes. Western hemlock stands were heavy on north aspects. Larch showed up strong on north slopes and terraces, while lodgepole pine occurred mostly on the sharper knolls or hog backs with south and west exposure.

After looking over the Deep Creek burn, it was decided to concentrate on a rectangular area roughly designated in Figure 1. A great advantage found in this selection was the possibility of detailed study of different aspects by means of a system of strips radiating from a central point.

Detailed records of field conditions and natural reproduction were obtained on chained compass strips. Counts of reproduction by species were made separately for each chain on a strip either 10 or 20 links in width. Seedlings the ages of which could not be definitely determined were merely tallied. Record was made of live and dead trees by species. All live trees near the strip or within plain view were also noted. It was impossible, without detailed study, to be sure in all cases of the species of dead trees, especially on the double burn. The records included species of vegetation present, the density of the vegetative cover from the standpoint of shading or competition, soil conditions, aspect, and degree of slope.

TRAIL CREEK AREA: BURNS OF 1910 AND 1870

This part of the 1910 burn is a wedge-shaped area of about 200 acres, lying south of Trail Creek and rising with regular slopes and uniform grades from 3,100 to 4,000 feet elevation, with northwest and northeast aspects (fig. 2). The timber here was more than 200 years old, very dense and tall, with a high percentage of white pine; the soil was moist, deep, and fertile. This permitted a study of natural reproduction on a clean and complete 1910 burn where no green trees survived the fire.

Another tract studied lies on a broad spur terminating at the junction of Trail Creek and Bear Creek (fig. 3). The somewhat uniform slopes, which rise from 3,100 feet to 3,600, are interrupted by several deeply cut draws. This affords local variations in aspect which show striking differences in flora and natural reproduction. Here it was that the mature forest—including western white pine, western larch, and Douglas fir—was largely destroyed about 1870 and where the dense and vigorous young forest attained a 40-year growth and was completely burned in 1910.

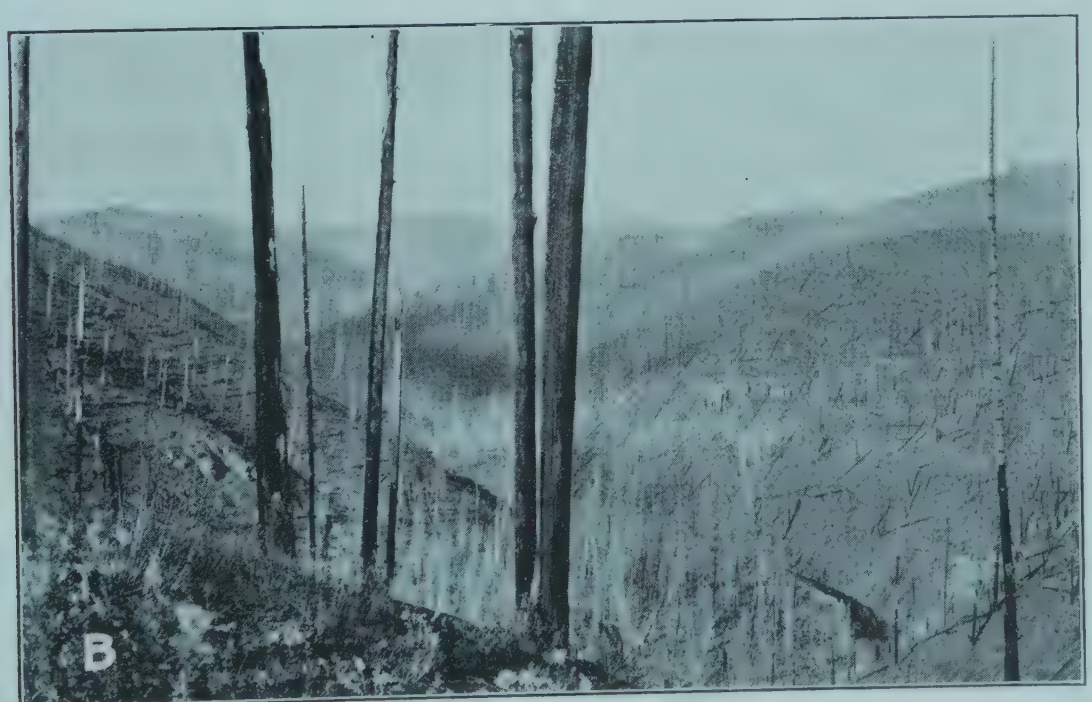
DOUBLE BURN: 1910 AND 1919

VARIOUS ASPECTS

The first point of attack was the double 1910 and 1919 burn on Deep Creek. Here the elbow of the river has formed a round peninsula which tapers up to a central point at 4,100 feet elevation, as shown in the smaller western section of the inclosed area in Figure 1. This offered an excellent opportunity for studying reproduction on different aspects.

Both the big fires of 1910 and 1919 struck this point very hard, making a fairly clean sweep of all live trees in

A. Looking west across Deep Creek, where two very severe fires (1910 and 1919) destroyed all living trees except some tall larches on the lower north aspect. Dotted lines show location of plots and courses of count strips



B. View north along Deep Creek from point between Deep Creek and Jordan Creek. Horizontal Prichard slate and steep lower slopes capped by the gentler intermediate slopes of the overlying Burke formation. The middle of the skyline is formed by the Idaho–Montana divide, toward which both the 1910 and the 1919 fires traveled

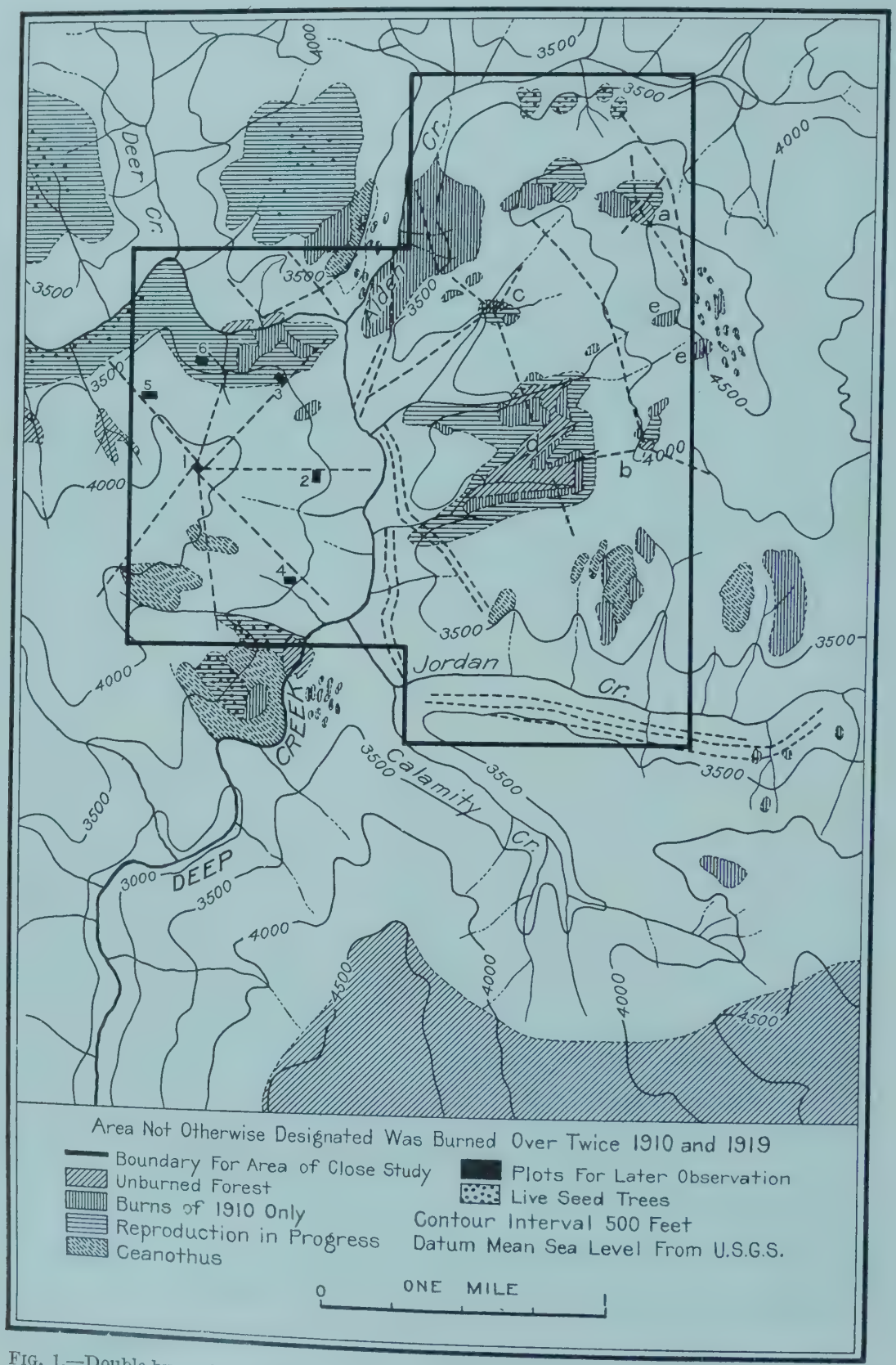


FIG. 1.—Double burn of 1910 and 1919 on Deep Creek area, Coeur d'Alene National Forest, Idaho

the first burn, and on the south and east aspects killing all trees except an occasional larch. On the north slopes, particularly the lower parts, several large western larch survived the 1910 burn but were killed in 1919. The north aspect also showed patches of reproduction which followed the 1910 fire and which escaped the 1919 fire.

Beginning at 4,100 feet elevation, seven radiating strips were run down to the river, some of them shown in

1. Insufficient natural reproduction on all aspects.
2. Deplorably small numbers of white pine everywhere.
3. Restocking up to 200 seedlings per acre of western larch on north aspect.

The western larch reproduction consisted of 2, 3, and 4 year old seedlings, the largest percentage by far being 4-year-olds which followed the 1919 burn. Seedlings of white pine occurring on the double burn were found to be 1, 2, and 4 years old. There was no

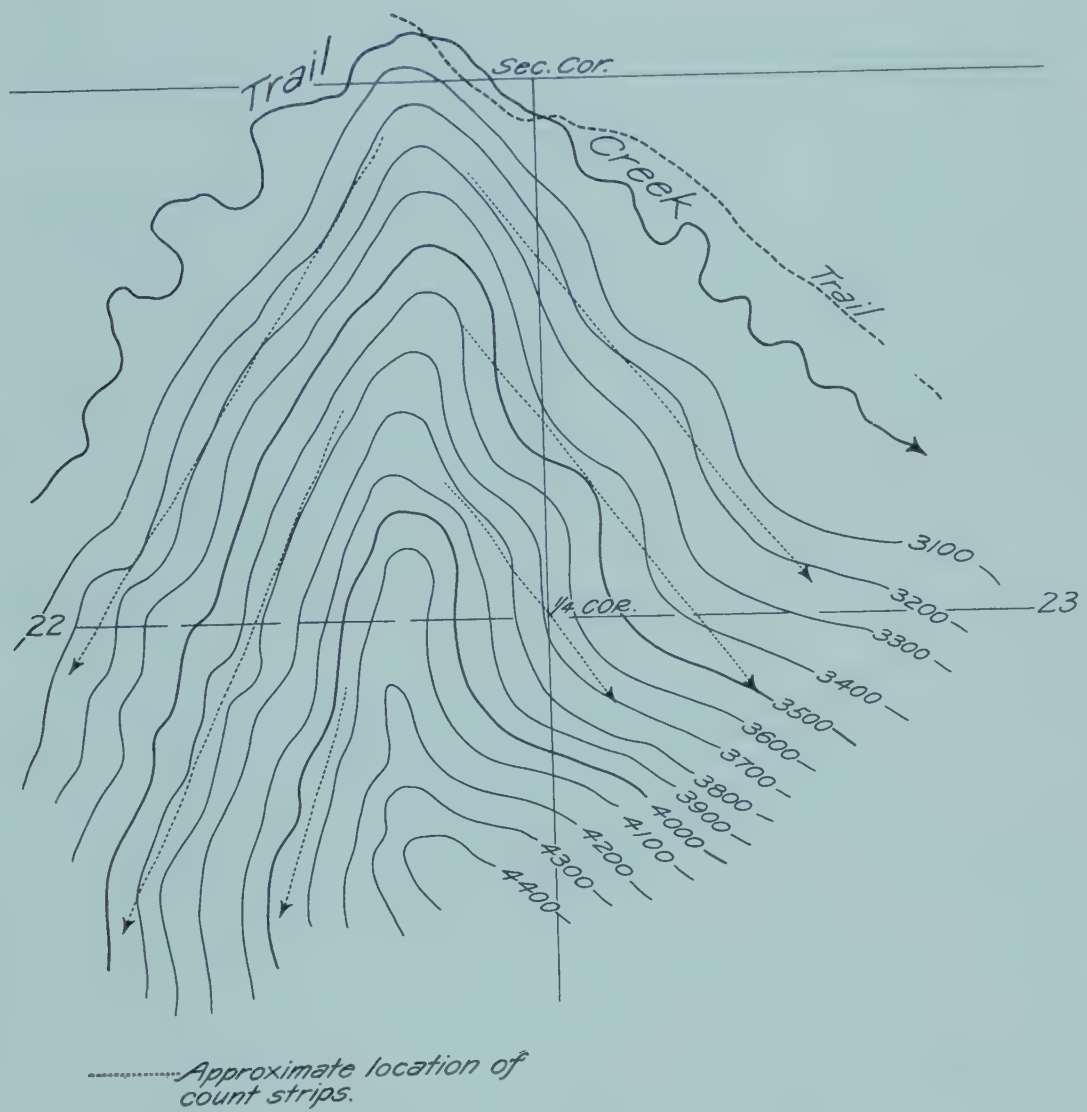


FIG. 2.—Map of portion of 1910 burn, Trail Creek

Plate 1, A. Reproduction was counted on these strips. In addition, six plots—one of one-tenth acre, the rest one-quarter acre—were established on the different strips at intermediate elevations. Four of these are shown in the illustration; all can be seen in Figure 1. The seedlings on the plots were counted and staked, with the results given in Table I. The reproduction on these plots will be examined in later years. The main deductions from Table I are:

evident relation between the original stand and the reproduction, except in the case of western larch.

The fact that 1-year-old seedlings of white pine, and occasional seedlings of Douglas fir, and even white fir, from 1 to 4 years old, were found, indicates strongly that a very sparse long-range seeding is in progress. If white pine only had been found it might have been assumed, as one possible explanation, that the seed had been scattered in 1919 at the time of the fire, and that

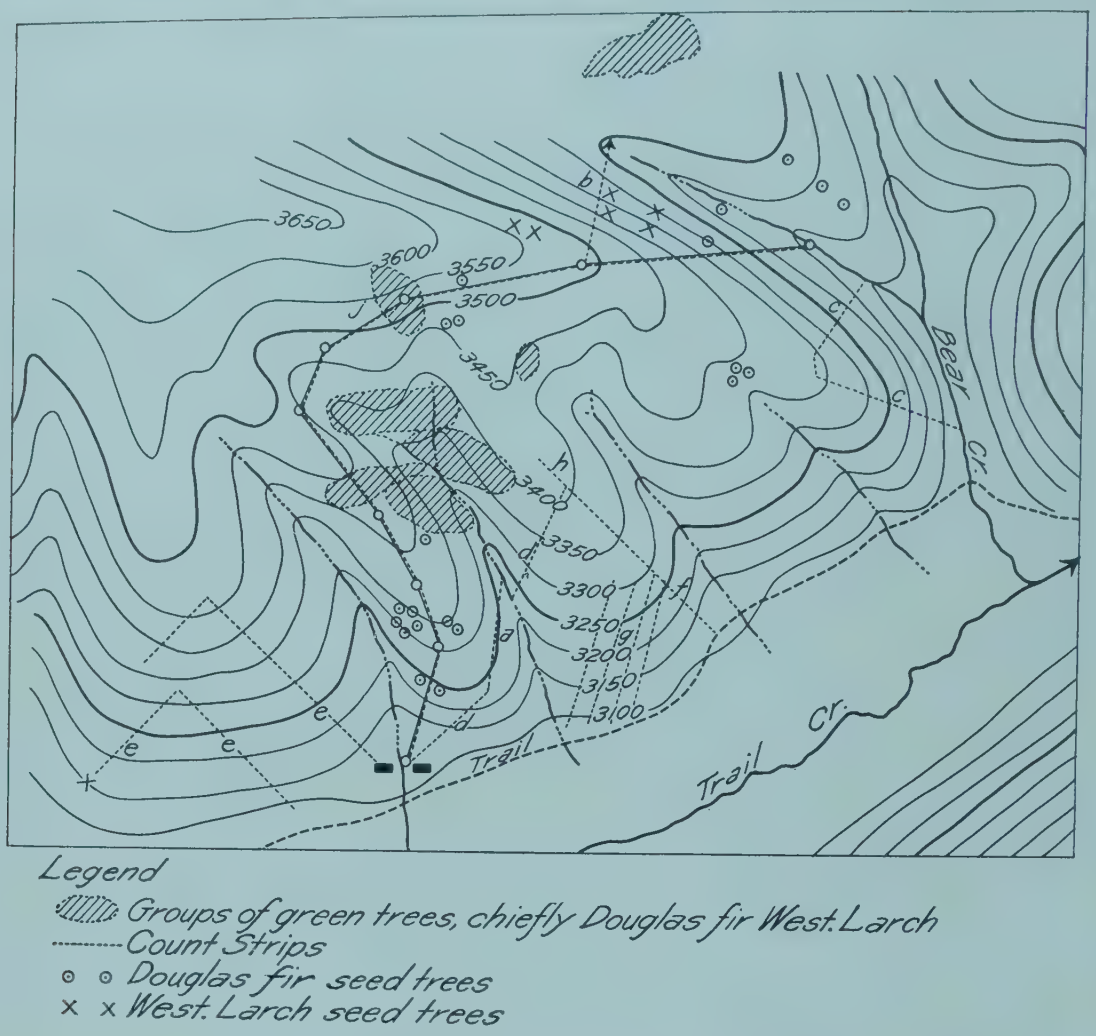
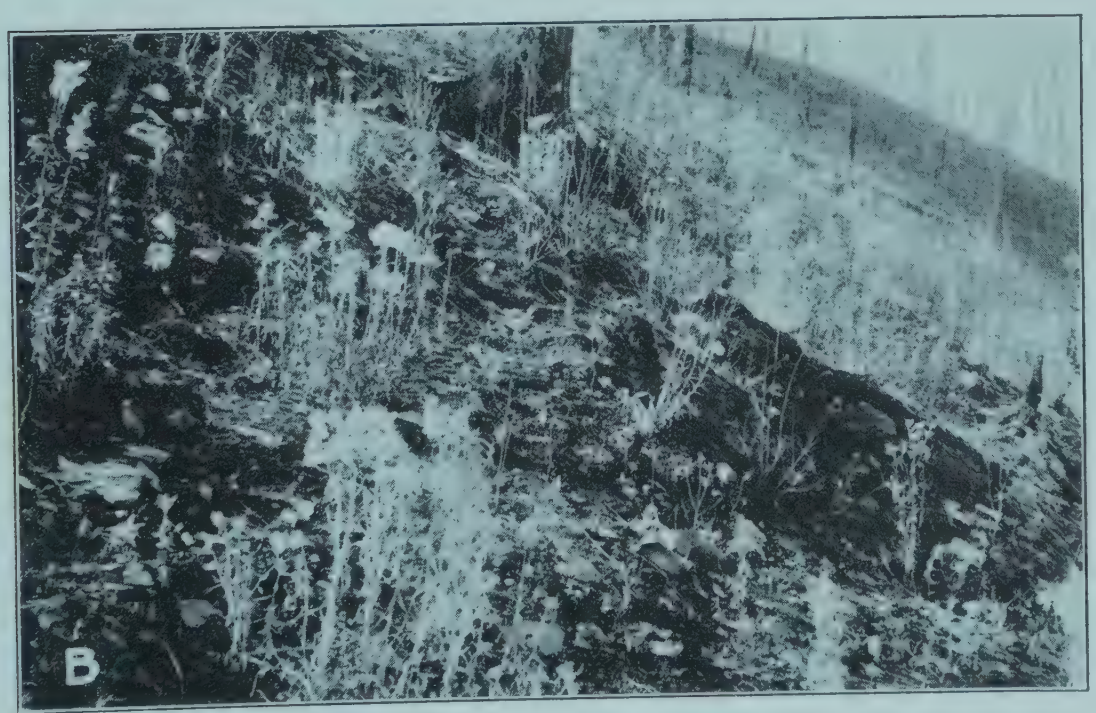


FIG. 3.—Map of portion of Coeur D'Alene National Forest

TABLE I.—*Double burn of 1910 and 1919: Reproduction count on permanent plots, all aspects; compared with average for strips*

Species	Seedlings per acre by plots								Seedlings by count strips	
	No. 1 top	No. 5 NW.	No. 6 N.	No. 3 NE.	No. 2 E.	No. 4 SW.	Average per acre	Percentage by species	Average per acre	Percentage by species
Western white pine	50	0	8	8	0	0	6.7	10	2.2	5
White fir	0	0	16	0	0	0	3.0	4	1.4	3
Western larch	20	12	168	104	8	16	58.5	83	36.4	84
Douglas fir	0	4	0	0	4	0	1.5	2	0	0
Western hemlock	0	0	0	0	0	0	0	0	1.3	3
Lodgepole pine	10	0	0	0	0	0	.7	1	1.3	3
Engelmann spruce	0	0	0	0	0	0	0	0	0.7	2
Alpine fir	0	0	0	0	0	0	0	0	0	0
Total	80	16	192	112	12	16	70.4	100	43.3	100
Area of plots (acres)	0.10	0.25	0.25	0.25	0.25	0.25				
Percentage of slope	10	25	60	60	45	45				

A. View east up Jordan Creek. Through the narrow canyon in the foreground both the 1910 and 1919 fires rushed to reach the upper basin. Natural reproduction is utterly inadequate



B. Near view on middle north slope, Deep Creek, showing complete destruction by the two hard burns of 1910 and 1919. Vegetation is mostly pearly everlasting. Reproduction plot No. 6 was located here

some of this seed had "delayed germination," but other species whose seed does not hold over are also present as 1-year-olds. This seems to be strong evidence of seed dispersion for distances of a mile or more.

South of Alder Creek is a long and steep north slope from 3,000 to 4,700 feet in elevation. This was heavily burned in 1910 and 1919, and all green timber was destroyed except a few larches on the lower part of the slope. Here the natural restocking is extremely irregular and entirely insufficient. Strangely enough, on this north slope no new white pine seedlings were found. The nearest white pine trees which could have furnished seed are part of a seed group remaining on top of a knoll less than one-half mile to windward. Occasional new Douglas fir and lodgepole pine seedlings were found, and these might well have come from this same seed-tree group. Seedlings of western larch 3 and 4 years old formed 88 per cent of the total, and unmistakably showed their relation to large parent trees surviving the 1910 burn and killed or dying as a result of the 1919 fire.

One of the most completely devastated slopes met with in this study is the north aspect toward Jordan Creek (pl. 2, B). Here was formerly a dense and mature forest containing much splendid white pine and some western larch, white fir, and hemlock. Both the 1910 and the 1919 fires raced up the narrow Jordan Creek canyon shown in the foreground of Plate 2, A, making an unusually clean burn. Even the stumps and roots of the trees were burned, in many places leaving nothing but holes to indicate where the trees had been. The rocks showed marked coloring as a result of the heat, and the soil is now apparently very poor and has deteriorated, as evidenced by unusual coloring, a large amount of rock material near the surface, and the short and scant vegetation. On a strip a mile long, covering an area of 4 acres, not more than 12 new seedlings were found. These were 3 white pines 2 years old, 2 white firs, 2 Douglas firs under 4 years, and 5 western larches 4 years old. On the north aspect, where the second fire had been less intense, were eight small patches of seedlings which had come up after the 1910 burn and which had escaped the 1919 fire. Here, as well as on the previous double-burned north slopes, natural reproduction is decidedly insufficient.

UPPER SOUTH AND WEST ASPECTS

The land directly to the east of Deep Creek slopes to the southwest from 4,700 feet at the top to 3,000 feet elevation at the river. The rise for the first 500 feet from the river is quite steep, then follows an intermediate benchlike slope interrupted by deep draws. Above 4,000 feet the land is again very steep. The slopes are uniform on the whole, and regular and free from débris. The soil is generally deep and fertile, but there are many barren and shallow stretches at the apexes of the several spurs and on the lower and steeper portions near the river. Both the 1910 and 1919 fires played great havoc with the lower and upper slopes but spared several bodies of green trees in the principal draws (fig. 1).

One count strip was run horizontally along the entire length of the upper southwest aspect, and another lower down, as shown on Plate 3, A. The counts are given in Tables II and III.

Table III shows a total of only 14 seedlings per acre on the upper south slope and 50 on the lower. As indicated in Table II, these seedlings are 1 to 4 years old. Only 14 per cent on the upper slopes are western white pine. This site is directly exposed to the sun and wind, so that seedlings, especially of the white pine and western larch, would be likely to dry out in great numbers, except those in the shade of logs or stumps. In fact, most of those found were where the slope swings to the northwest.

TABLE II.—*Reproduction count by age classes on parallel strips, double burns of 1910 and 1919, upper and middle southwest aspects*

Age class	Upper strip, seedlings per acre		Lower strip, seedlings per acre	
	Number	*Per cent*	*Number*	*Per cent*
1 year old	0	0	4	8
2 years old	4	29	4	8
3 years old	6	42	4	8
4 years old	4	29	38	76
Total	14	100	50	100

TABLE III.—*Reproduction count by species on parallel strips, double burns of 1910 and 1919, upper and middle southwest aspects*

Species	Upper strip			Lower strip		
	Dead trees	Seedlings per acre		Dead trees	Seedlings per acre	
	Per cent	*Number*	*Per cent*	*Per cent*	*Number*	*Per cent*
Western white pine	25	2	14	44	19	38
White fir	5	7	50	6	0	0
Western larch	16	3	22	21	31	62
Douglas fir	42	2	14	0	0	0
Western hemlock	12	0	0	26	0	0
Engelmann spruce	0	0	0	3	0	0
Total	100	14	100	100	50	100

LOWER SOUTH AND WEST ASPECTS

The lower south and west slopes are, as stated, much steeper than the middle or upper. The soil is thin, vegetation sparse, and rock material much in evidence. (Pl. 3, B.) Both fires burned very hard, leaving no green trees. Close examination was made of the southerly aspects on Deep Creek and some distance up Jordan Creek, but except for an occasional western larch seedling growing in a shaded place no reproduction could be found. Some seeds very likely fell on these slopes after the 1919 burn, but if any did they probably met with such adverse conditions of heat and drought that any seedlings that came up died during the first and second seasons after germination, which would be expected in the light of the results of studies made at the Northern Rocky Mountain Forest Experiment Station.

BOTTOMS

Reproduction on the bottoms, river flats, or flood plains along the main creeks has not been investigated to any great extent. Conditions differ greatly on such sites, making classification very difficult, for there are variations all the way from moist, deep, loamy soil, covered with a wealth of grasses, to thin, shallow soil or pure gravel. The original stand of trees also varies greatly. On the Deep Creek bottoms there has been considerable spruce, white fir, and white pine. It may safely be said, however, that good reproduction on these sites has followed the single 1910 fire and not the second fire, except in the vicinity of seed trees.

BENCHES

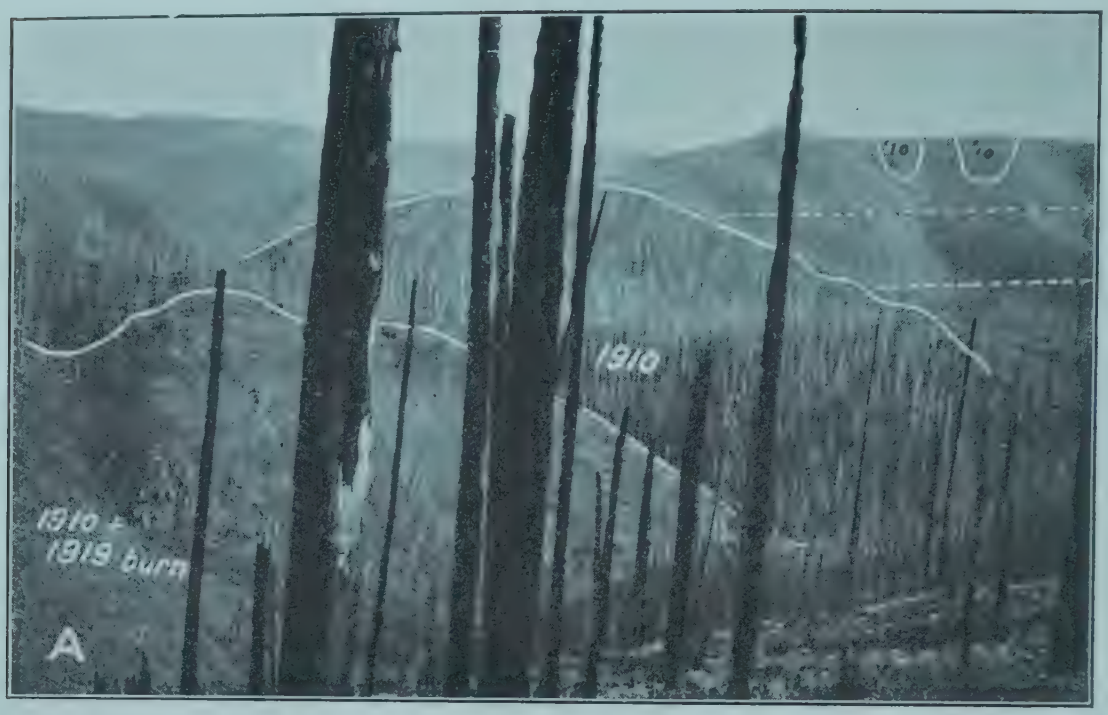
The benches or high terraces are those lying between the 3,500 and 4,000 foot contours. Here the first burn of 1910 killed all species except western larch, the larch being left irregularly distributed over the area. The larches seeded and restocked abundantly after the first fire, as witnessed by small patches of reproduction which escaped the later 1919 burn. Many of the thick-barked larch seed trees survived both fires and are now furnishing seed for restocking.

TOPS AND KNOLLS

One very pronounced characteristic, which has appeared repeatedly on both the Deep Creek and the Trail Creek areas, is the greater numbers of lodgepole pine seedlings occurring in fairly solid bodies on dry southerly points and on thin soil between the lower steep south or west slopes and the terraces. Seed-bearing lodgepole pine was present on these sites in goodly number before the 1910 burn and seeded in well afterwards, and wherever this reproduction has survived the 1919 conflagration it presents a solid mat of lodgepole saplings about 10 to 13 years old, usually with a smaller number of shorter white pine seedlings included. The knolls and ridge tops which burned hard in both 1910 and 1919 show no reproduction, but many such spots escaped one fire, and in some cases both fires.

INFLUENCE OF SEED GROUPS

It is now necessary to examine the records of seed dispersion from groups of trees which by reason of the influence of topography in checking or abating the fury of a fire at certain points survived both the 1910 and 1919 fires. Such groups usually occur on high, sharp ridges, on very rocky ground, in deep sharp draws, or on moist bottoms. A study was made of



A. View east across Deep Creek to the double burn of 1910 and 1919 on southwest aspect. Seed group of western larch is to be seen on a ridge to right of center; remnants of the 1910 burn on left and double-burn south aspect below. Horizontal dotted lines mark count strips

B. Near view of steep lower south aspect, Jordan Creek, showing effect of soil leaching and dense grass vegetation; natural reproduction nil. Though seed would fall here by long-range dispersion, the seedlings would be at a serious disadvantage

dispersion of seed from four such seed groups, which appear as *a*, *b*, *c*, and *d* on the map, Figure 1, and also in Table IV.

Group A is a high and very prominent group and is shown on the elevation in the background of Plate 3, A. The greatest number of trees in the group are western larch, but there are also a few live Douglas firs, alpine firs, and white pines. Briefly, restocking to the north of the seed group is only one-third complete and to the south and west is also insufficient.

The distances to which seed is dispersed are from 9 to 11 chains on the north to 5 to 6 on the south side of the seed trees. The dispersion for the greater distance in the northerly direction evidently is promoted by the prevailing wind and a more favorable aspect. White pine and Douglas fir seeded to a distance of 9 chains from the parent trees and white fir and western larch to 11 chains. Restocking in the northerly direction is also more regular than toward the south, in that it shows a gradual falling off in numbers

TABLE IV.—*Effective seed dispersion from groups of live trees, double burns of 1910 and 1919, 1923* [a]

Species	Greatest distance of seed distribution from parent trees, and average number of seedlings per acre, by seed-tree groups and count strips											
	Seed-tree Group A								Seed-tree Group B [b]			
	NW. strip		NE. strip		SE. strip		SW. strip		NE. strip		NW. strip	
	Distance	Seedlings	Distance	Seedlings	Distance	Seedlings	Distance	Seedlings	Distance	Seedlings	Distance	Seedlings
	Chains	*Number*	*Chains*	*Number*	*Chains*	*Number*	*Chains*	*Number*	*Chains*	*Number*	*Chains*	*Number*
Western white pine	9	36	7	225	2	30	3	35	-------	-------	4	10
White fir	11	5	5	30	-------	-------	-------	-------	9	15	7	20
Western larch	11+	86	8+	70	1	12	5	40	8	5	-------	-------
Douglas fir	9	36	3	15	-------	-------	-------	-------	7	480	6	315
Western hemlock	7	14	3	5	-------	-------	-------	-------	-------	-------	6	25
Lodgepole pine	1	5	4	15	4	450	6	165	-------	-------	-------	-------
Alpine fir	2	9	2	180	-------	-------	-------	-------	-------	-------	-------	-------
Total	-------	191	-------	540	-------	492	-------	240	-------	500	-------	370
		Per cent		*Per cent*		*Per cent*		*Per cent*		*Per cent*		*Per cent*
Western white pine	-------	19	-------	42	-------	6	-------	15	-------	0	-------	3

Species	Greatest distance of seed distribution from parent trees, and average number of seedlings per acre, by seed-tree groups and count strips									
	Seed-tree Group C [c]				Seed-tree Group D					
	NW. strip		SE. strip		NE. strip		NW. strip		SE. strip	
	Distance	Seedlings	Distance	Seedlings	Distance	Seedlings	Distance	Seedlings	Distance	Seedlings
	Chains	*Number*	*Chains*	*Number*	*Chains*	*Number*	*Chains*	*Number*	*Chains*	*Number*
Western white pine	2	15	9	19	3	5	5	21	6	5
White fir	11	10	-------	-------	7	50	5	112	6	10
Western larch	7	40	8	31	5	45	9	75	8	15
Douglas fir	7	5	-------	-------	5	10	3	71	6	5
Western hemlock	7	30	-------	-------	5	35	-------	-------	6	25
Total	-------	100	-------	50	-------	145	-------	279	-------	60
		Per cent		*Per cent*		*Per cent*		*Per cent*		*Per cent*
Western white pine	-------	15	-------	38	-------	3.4	-------	7.5	-------	8

[a] Slope varied for the different seed-tree groups and strips, as follows: Group A—NW. strip 25 to 50 per cent, NE. strip 25 to 30 per cent, SE. strip 20 to 30 per cent, SW. strip 30 to 55 per cent; Group B—30 to 50 per cent; Group C—NW. strip 10 to 25 per cent, SE. strip 25 to 35 per cent; Group D—NE. strip 30 to 50 per cent, NW. strip 20 to 35 per cent, SE. strip 0 to 20 per cent.

[b] No seedlings on southeast or southwest strips.

[c] No seedlings on northeast strip.

of seedlings per acre for given distances away from the parent trees. Many lodgepole seedlings occurred on the southerly strips, but these are not so much the result of dispersal from the seed groups as from the trees which existed in situ previous to the fires.

Another interesting seed group (marked *b* on the map and in Table IV) was found on a southerly spur at 4,000 feet elevation. This group consists of Douglas fir only, about 100 years old, rather short but of fairly good crown, and much deteriorated by the exposure resulting from fire. From this little group of a half-dozen trees count strips were run in four directions. The location of the group is such that the prevailing wind would carry the seed to a northwest aspect where chances of seedling survival would be better than on the south side.

In four years restocking to the northeast of the parent trees has taken place at the rate of 500 seedlings per acre; and to the northwest, 370 per acre, and this to a distance of 9 chains or less. There is absolutely no restocking to the southwest or southeast. Douglas fir seedlings predominate and show unmistakable relations to parent trees both in age and distribution; the white fir and the larch seedlings evidently came from trees which died during or shortly after the fire.

A third group of green seed trees (marked *c* on the map, and in Table IV) was found in a draw. This group consisted of five large white fir trees; four white pines, only one of which showed cones; three spruces, rather small but with many cones; and several small hemlocks. The location was such that seed dispersed toward the north and east would fall upon a very dry site. Count strips were run northwest and southeast from this group. The results are given under Group C in Table IV. Irregular restocking has taken place northwest and southeast to a distance of about 9 chains and none at all toward the northeast. What has come in is as yet insufficient, only 50 and 100 seedlings per acre.

A fourth group of seed trees, much larger and of older timber than any already described, occurs to the south (marked *d* on the map and in Table IV). The trees are western larch, western hemlock, white fir, Douglas fir, and a very few western white pines, all overmature and very tall. The seed from these trees would fall on the warm lower south slope toward the east and northeast, and on the terrace, or in the draw. Count strips were run in three directions to observe restocking toward the northwest, northeast, and southeast. The counts are given under Group D in Table IV.

Restocking from this group may be said to have only begun. The count shows 60 seedlings per acre toward the southeast, 145 toward the northeast, and 279 toward the northwest. Here, evidently, the rôle which aspect plays in the survival of seedlings is less pronounced than in the case of the previous seed groups. The ground is not as steep as it is at Groups B and C, and appears more favorable for seedling establishment.

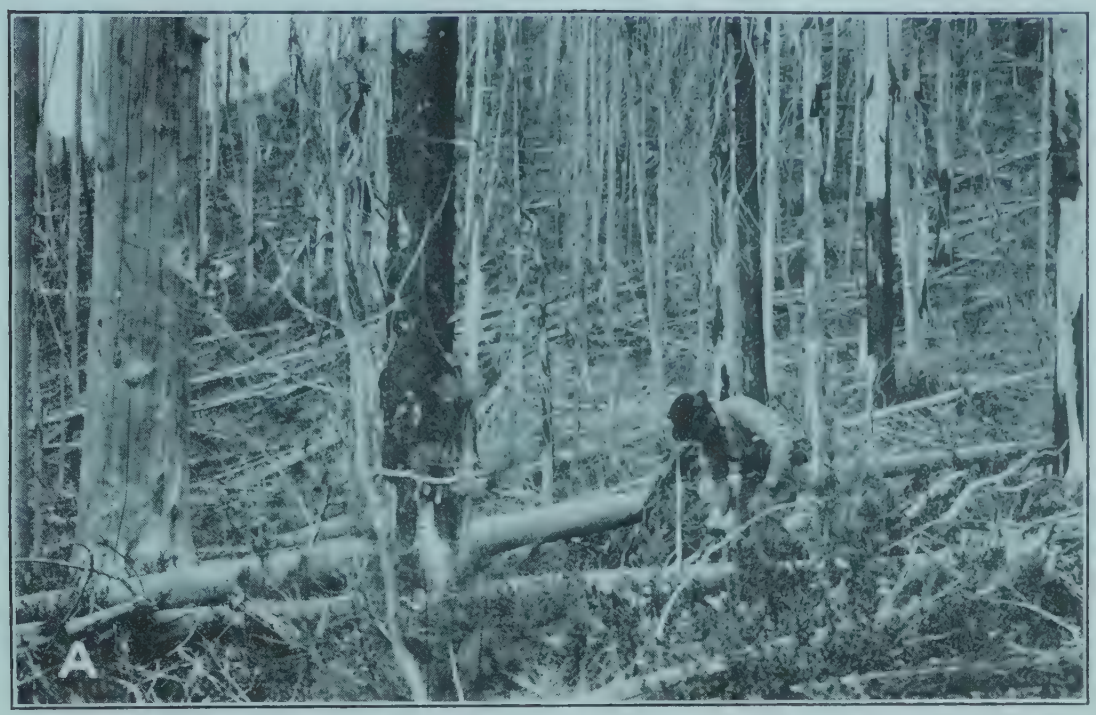
SINGLE BURNS OF 1910

DEEP CREEK BURNS

An area of less than 160 acres on the northwest aspect near the mouth of Alder Creek was burned in 1910 but escaped the second (1919) fire. Here had been a full and overmature stand containing much splendid white pine, as well as larch, western hemlock, white fir, Douglas fir, and a small percentage of lodgepole pine, spruce, and alpine fir (pl. 4, A). The soil is deep and of good quality. The fire of 1910 left not one live tree on the entire area. It does not seem possible that any of these trees could have been the source of cones or seed subsequent to 1910, though a few may have had cones and seed at the time of the burn. On this area two long count strips were run diagonally down the slope toward the creek. The counts are given in Tables V and VI.

Table VI, for this northwest slope, shows 265 seedlings per acre, 37 per cent of which are western white pine—not a heavy stand, but a healthy and vigorous one. Table V classifies white pine in two age classes, one class from 8 to 13 years and a second at 4 years. For want of conclusive data, which could come only from consecutive examinations begun shortly after a fire and followed through for a number of years, the source of the seed can not be stated positively. The hypothesis that the seed was in the duff or on the burned trees may still hold. It is difficult to believe that the seed which gave rise to the older seedlings came from the outside, for the supply seems to have been cut short exactly five years after the 1910 fire. An outside source would presumably have been cut short nine years later by the 1919 fire.

Even more speculative is conjecture regarding the source of the 4-year-old white pine seedlings. Why 4 years, no



A. Near view of 1910 burn, Alder Creek. White-pine seedlings number 265 per acre, some of which may be seen in the foreground. The standing and down trees furnish some shade and shelter to the seedlings, which is very desirable. Unfortunately, the fire hazard is very great

B. Erosion in gully on lower part of north aspect, Alder Creek, burned hard in 1910 and 1919

TABLE V.—*Reproduction count, by age classes, single burn of 1910, Deep Creek, northwest aspect, 1923*

Age class	All species	White pine
	Per cent	*Per cent*
1 year		
2 years	0.5	
3 years	1.0	
4 years	8.0	58.0
5 years	.5	
6 years	1.5	
7 years	3.5	
8 years	18.0	50.3
9 years	16.0	50.0
10 years	30.0	43.7
11 years	11.5	5.5
12 years	3.5	
13 years	6.0	20.0
Total	100.0	36.6

years old and older, and bears a close relation in species to the former stand. There are no new seedlings and no seeding is in progress. These areas were hard burned in 1910 and all the trees were killed. The nearest green trees on the windward side are one-half mile away. On the upper portion of the slope three western yellow pine seedlings were found, all growing in one spot. These were the only evidence of the species found on the Deep Creek area. Since the first fire much snow brush (*Ceanothus velutinus*) has taken hold, and considerable grass sod now covers from 80 to 90 per cent of the surface and is considered a hindrance to natural restocking. Reproduction counts of this slope are given in Table VI.

TABLE VI.—*Reproduction count, by species, single burn of 1910, Deep Creek, northwest and upper southwest aspects, 1923*

Species	Northwest aspect			Upper southwest aspect	
	Former matured stand	Seedlings per acre		Seedlings per acre	
	Per cent	*Number*	*Per cent*	*Number*	*Per cent*
Western white pine	27.0	97	36.6	38	18.7
White fir	7.5	25	9.4	91	44.8
Western larch	1.5	88	33.2	3	1.5
Douglas fir	2.2	10	3.8	6	3.0
Western hemlock	39.0	37	14.0	23	11.3
Lodgepole pine	21.8	4	1.5	42	20.7
Engelmann spruce	.5	4	1.5		
Alpine fir	.5				
Total	100.0	265	100.0	203	100.0

more, no less? This takes us to 1919. Did the 1919 conflagration scatter seed? If so, it was distributed in advance of the fire, for no 4-year-olds appear on the double burn. As the 4-year-old seedlings were not so evident elsewhere on single burns, the source and dispersion of the seed which produced them must have been local rather than general. It seems reasonable to suppose that this seed was carried from green trees located in draws a half or a quarter of a mile to the south.

Observations and counts made in the 1910 burn on an upper southwest slope, Deep Creek, at elevations ranging from 4,300 to 4,500 feet, cover the area marked *e* on the map in Figure 1. In this area are two large patches on which the previous stand was 100 to 125 year old white pine, Douglas fir, lodgepole pine, lowland fir, and alpine fir. The natural reproduction is 8

TRAIL CREEK BURNS

On the southwest corner of the large 1910 burn a spur terminating at the elbow of the Trail Creek was studied. This is almost cone shaped and more than 1,000 feet high. The principal slopes are generally northwest and northeast. (See fig. 2.) Previous to the 1910 burn there was an excellent stand of mature timber here which contained a high percentage of white pine. On the northeast slope the stand contained principally white pine, hemlock, and larch; and on the northwest white fir, Douglas fir, and scattered spruce and alpine fir in addition. All species, except some big larches on the lower part of the northeast aspect, were destroyed by the fire. Every part of the surface was burned. The soil is deep and of good quality, except on the upper northwest aspect and on the

ridge which points toward the sharp bend of the creek. Three horizontal count strips were run on the northwest and three on the northeast slope, covering the upper, middle, and lower parts by separate tallies. The counts have been summarized in Tables VII and VIII.

Natural restocking is, in most cases, less than 200 seedlings to the acre, this number being exceeded on the lower northeast slope only. This is much poorer than anticipated. It is encouraging, however, to note that white pine shows a higher percentage in the reproduction over that of the previous stand.

The age classes of the seedlings show that the greater number are more than 7 years old, indicating that these older ones may have come from seed in the soil or duff. The better restocking found on the lower part of the northeast slope contains many seedlings from 1 to 4 years old. These appear where much typical vegetation such as accompanies white pine forests occurs, as species of Tiarella, Coptis, Asarum, etc., and where the soil is deeper and and more moist than on the southwest slopes.

Most of this seed came from green trees now extant on the flat toward the eastern portion of the area. The source of the miscellaneous younger seedlings found promiscuously over the area is most likely the old unburned forest to the southwest. Fresh and clean seed coats found underneath several 1-year old seedlings of white pine, hemlock, and Douglas fir point to dissemination of the seed by the wind and not storage in the soil. The exact relation of this dispersion of seed to green timber, has not been traced out, and as far as the strips were run toward this green timber no migration or relation was made evident.

TABLE VII.—*Reproduction count, by age classes, single burn of 1910, Trail Creek, northwest and northeast aspects, 1923*

Age class	Northwest aspect—Seedlings per acre						Northeast aspect—Seedlings per acre					
	Upper slope		Middle slope		Lower slope		Upper slope		Middle slope		Lower slope	
	No.	*Per ct.*	*No.*	*Per ct.*	*No.*	*Per ct.*	*No.*	*Per ct.*	*No.*	*Per ct.*	*No.*	*P. ct.*
1 year					3	1.5			3	2.0	33	3.0
2 years	3	4.0	1	1.0	3	1.5	1	0.5	8	5.0	60	5.0
3 years	4	5.0	3	2.0	1	.5	2	1.0	1	.5	6	1.0
4 years	7	8.0	9	7.0	20	11.0	10	5.0	11	8.0	73	7.0
5 years	1	1.0	5	4.0			2	1.0	5	3.5	60	5.0
6 years	3	4.0	2	1.0	13	7.0	1	.5	15	10.0	40	4.0
7 years	17	20.0	4	3.0	14	8.0	2	1.0	15	10.0	180	16.0
8 years	31	37.0	25	19.0	29	16.0	19	9.0	25	17.0	200	18.0
9 years	7	8.0	20	15.0	24	13.0	21	10.0	9	6.0	155	14.0
10 years	4	5.0	9	7.0	17	9.0	26	12.0	21	14.0	173	15.0
11 years	5	6.0	10	8.0	10	6.0	27	12.0	14	10.0	60	5.0
12 years	2	2.0	3	2.0	1	.5	17	8.0	3	2.0	27	2.0
13 years			1	1.0			4	2.0				
Unclassified			39	30.0	46	26.0	81	38.0	17	12.0	53	5.0
Total	84	100.0	131	100.0	181	100.0	213	100.0	147	100.0	1,120	100.0

TABLE VIII.—*Reproduction count by species, single burn of 1910, Trail Creek, northwest and northeast aspects, 1923* [1]

Species	Percentages of seedlings and dead trees, NW aspect					Percentages of seedlings and dead trees, NE aspect				
	Upper slope	Middle slope	Lower slope	Average	Dead trees	Upper slope	Middle slope	Lower slope	Average	Dead trees
Western white pine	44	50	63	52	26	50	50	32	44	28
White fir	6	13	4	8	7	6	11	5	7	10
Western larch	1	4	3	3	1	5	4	8	6	2
Douglas fir	1	2	1	1	5	2	4	17	8	3
Western hemlock	29	18	24	24	51	29	28	25	27	53
Lodgepole pine	15	11	2	9	1	3	3	12	6	
Engelmann spruce	4	2	3	3	9	4		1	2	
Alpine fir			1			1				2

[1] Percentages are given to nearest unit; total is thus not always an even 100 per cent.

It was, however, definitely shown on the northwest slope that a great many more seedlings of the older age classes existed on the minor northerly exposures, such as occur near draws, than on the general west or southwest aspects, and that on such northerly aspects much of the typical forest flora which is seen under the virgin forest has survived. This indicates clearly that there was abatement of the intensity of the burn, with less damage to soil and site conditions on the protected than on the exposed aspect, an important factor in restocking from seed in the duff.

An upper northwest and west aspect in the 1910 burn at an elevation of over 4,000 feet, and above the three lower strips, was also examined. Before 1910 this had been mainly a Douglas fir type, such as is often found on steep upper aspects in Idaho and western Montana. All of the trees had been killed outright in 1910. From both the count strips and general observation, it was concluded that this area neither has been nor is now restocking. Only a few lodgepole pine seedlings were found. The reasons could be sought in absence of seed, as well as in exposed site with extreme drought and heat in summer, or in heavy sod competition. It seems reasonable to suppose that all of these factors are here strong hindrances to natural restocking.

DOUBLE BURN OF 1870 AND 1910

About 1 mile west from Magee Ranger Station is the area already mentioned that was burned hard about 1870 and again in 1910. This is a point of land between Trail Creek on the south and Bear Creek on the east, rising from 3,000 to 3,500 feet and more in elevation, broken to the south by several sharp draws, and many steep, bare, or grassy south slopes. (See fig. 3.) Previous to 1870 there was a dense overmature stand of the usual mixture of white pine, white fir, western larch, Douglas fir, etc., the Douglas fir being most prominent on the southerly aspect, the larch and lodgepole pine on the tops and white pine, white fir, and hemlock on protected aspects. On this area count strips were located according to aspects as shown by the dotted lines in Figure 3. The map shows the approximate location of green trees as well as the position and direction of the count strips.

After the first fire natural reproduction was generally good, even on the south and west aspects, and the resulting young stand, which was killed in 1910, contained much white pine.

As a result of the 1910 burn, this area presents three major and distinct field conditions induced by differences in topography, with the character and age of the forest approximately uniform for all three. Discussion of reproduction can well be divided accordingly into northerly aspects, southerly aspects, and tops and benches. On account of the lateness of the season, the reproduction studies on the higher points were not completed.

NORTH AND EAST ASPECTS

Tables IX and X under strip A present data from a strip run in a northeast direction from the top of the spur (point *a* fig. 3) down into the draw, a distance of 5 chains. The purpose was to find out what reproduction had taken place where no seed trees had been either before or after the 1910 burn. Previous to 1910 a dense and thrifty young stand of white pine, western larch, Douglas fir, and white fir had come up here. Practically all of these were down in 1923.

Table X shows a total of 280 seedlings per acre, of which 50 per cent are white pine. There are practically no seedlings younger than 7 years. The distributions of the seedlings do not show any relation or order which would indicate the source of the seed or the migration. There are no live trees immediately to the south and west and no indications of seed dispersion.

A second and even more representative north aspect of this double burn is that covered by strips *b* and *c*, Figure 3, a steep slope where all trees but a few big larches were killed in 1870. These survivors and the young, thrifty 40-year stand surrounding them were all killed in 1910. Reproduction counts are given under strip *b* and strip *c* in Tables IX and X.

Observations at strip *b* show a total of 1,313 seedlings per acre, with 23 per cent white pine; and at strip *c* 535 seedlings per acre, of which 33 per cent are white pine. Seeding both of white pine and the other species appears from the distribution by age classes to have been in progress ever since the 1910 fire, and it is now impossible to know which of these sprang from seed in the duff and which did not. At any rate, the restocking and the condition of the soil and site after two bad fires are very gratifying.

TABLE IX.—*Reproduction count by age classes, double burns of 1870 and 1910, three strips, northerly aspect, 1923* [a]

Age class	Strip A—Seedlings per acre		Strip B—Seedlings per acre		Strip C—Seedlings per acre	
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
1 year			14	1		
2 years			19	1		
3 years			24	2	11	2
4 years	20	7	38	3	35	7
5 years			67	5	30	6
6 years			100	8	65	12
7 years	80	28	138	10	35	7
8 years	60	22	180	14	41	7
9 years	20	7	104	8	47	9
10 years	60	22	156	12	18	3
11 years			152	11	70	13
12 years			73	6	30	6
13 years	40	14	73	6	12	2
Unclassified			175	13	141	26
Total	280	100	1,313	100	535	100

[a] Percentages are rounded off to nearest unit.

TABLE X.—*Reproduction count by species, double burns of 1870 and 1910, three strips, northerly aspect, 1923* [a]

Species	Strip A—Seedlings per acre		Strip B—Seedlings per acre		Strip C—Seedlings per acre	
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Western white pine	140	50	305	23	176	33
White fir	40	14	85	7	41	7
Western larch			315	24	29	5
Douglas fir	60	22	33	3	24	5
Western hemlock	20	7	343	26	106	20
Lodgepole pine	20	7	175	13	135	25
Engelmann spruce			57	4	24	5
Alpine fir						
Total	280	100	1,313	100	535	100

[a] Percentages have been rounded off to the nearest unit.

SOUTH AND WEST ASPECTS

The figures for the south and west aspects on this double 1870 and 1910 burn tell quite another story. Tables XI and XII, which summarize data for strip *d*, show only 58 seedlings per acre. Only 12 per cent of these are white pine, while 54 per cent are lodgepole pine; and practically no seedlings are younger than seven years. Three other separate observations on these steep south and west lower slopes at *e*, *f*, and *g* show no reproduction except a few lodgepole pines. These bare aspects are typical of many other similar areas in the Magee district. They are the result of hard double burns, and are characterized by thin, rocky soil, much sod, grass, and patches of snow brush. In some cases Douglas fir trees, either singly or in groups, have survived the two burns, but they have not given rise to any stocking whatever. Natural reproduction here during 13 years has been practically nil. The double burns have, therefore, brought about these permanent sores—sites extremely difficult to reforest, either naturally or artificially. We may assume that similar unfortunate conditions will follow on the steep south and west aspect on Deep Creek and Jordan Creek.

TABLE XI.—*Reproduction count by species, double burns of 1870 and 1910, southerly aspect, 1923*

Species	Seedlings per acre.	
	Number	*Per cent*
Western white pine	7	12
White fir	7	12
Western larch	3	5
Douglas fir	10	17
Lodgepole pine	31	54
Total	58	100

TABLE XII.—*Reproduction count by age classes, double burns of 1870 and 1910, southerly aspect, 1923*

Age class	Seedlings per acre	White pine
	Number	*Per cent*
1 year		
2 years		
3 years		
4 years	3	100
5 years		
6 years		
7 years	7	57
8 years	3	
9 years	7	
10 years	14	
11 years	14	
12 years	7	
13 years	3	
Total	58	12

TOPS AND BENCHES

Studies of natural reproduction on the tops and ridges throughout the Deep Creek area indicate restocking chiefly to lodgepole pine. One tally shows the following per acre:

	Total per acre	Per cent
WWP	77	7.7
WF	38	3.8
WL	69	6.9
DF	15	1.5
WH	31	3.1
LPP	770	77.0
Total	1,000	100

This shows a large predominance of lodgepole pine, but a few white pine seedlings appear here and there underneath the lodgepole saplings. The ages are in general 7 to 13 years, indicating little or extremely scant seed dispersion during the last five years.

A study of the migration of western larch toward the south and southwest from a group of green western larch at *j* on the top of the ridge shows effective seed dispersion to a distance of 8 chains from the live trees following the 1910 burn, with a total of 870 seedlings per acre, 6 per cent of which are white pine and 55 per cent western larch. The ages of the larch seedlings range from 1 to 13 years. This phase of the study was not completed for the 1870 and 1910 burns.

DOUBLE BURN 1889 AND 1910

UPPER SOUTH AND WEST ASPECTS

The reproduction study of this area is far from complete. The work done has, however, helped to clear up certain points as to the origin of the present conditions and the causes responsible for similar results elsewhere.

On the upper south and west aspects along the Idaho-Montana Divide occurred two very hard burns in 1889 and 1910. The first, which resulted from the "Chloride boom" near Pend Oreille Lake, appears to have crossed the divide from the Montana side and to have met the prevailing wind and stopped in the dense forest at an elevation of about 4,000 feet. The 1910 fire approached with considerable speed from the south and west, destroyed the splendid reproduction which had started since 1889, and crossed the divide into Montana.

This double burn is interesting, both from the standpoint of reproduction and of succession, in that barely enough time elapsed for seed production on the young stand before the second fire and also because of a widespread growth of dense snow brush.

Previous to the 1889 fire there was a mature and well-developed stand here, chiefly western larch and Douglas fir, with some white pine and white fir. Douglas fir showed up strongest on the dry sites. Some of these firs and a few of the larches have survived both fires and now remain as small scattered groups or isolated single trees, but entirely too few to restock the area adequately.

A tally made on an area of 1.56 acres gave the following data per acre:

	Seedlings	Per cent
WWP	4.5	15
WF	3.5	12
WL	13	45
DF	4	14
WH	2	7
LPP	2	7
ES	0	0
AF	0	0
Total	29	100

A total of 29 seedlings per acre gave 15 per cent white pine. These pines were from 8 to 12 years old, and were

underneath the spread of the limbs and among the dense ceanothus and willow brush. They seemed healthy, though not vigorous, and showed promise of survival and growth. Evidently on this warm, dry site the shrubbery is to them a help rather than a hindrance. No seeding appeared to have taken place in the last 5 years.

The finding of these seedlings here was a matter of much encouragement in that they indicate the probability that these brushy areas will eventually come back into forest. It will be about 30 years, however, before those already established begin to seed effectively. After that, natural restocking should be complete in about 20 years. Thus these two burns retarded the establishment of a complete new stand for at least 50 years.

AGES OF WHITE PINE SEEDLINGS FOUND ON 1910 BURNS

A closer analysis of the time and manner of restocking of western white pine can best be made from the data obtained on the various 1910 burns. Study shows, in most cases, an active germination during the third, fourth, fifth, and sixth years, followed by an irregular germination in other years. In no instance has the germination of western white pine been pronounced during the first two years following the burn. White pine germination began in earnest in 1913, three years after the fire. This first active period culminated in 1915 on the 1870 and 1910 burn; in 1916 or 1917 on Alder Creek [2], and in 1917 and 1918 on the Trail Creek areas.

It may occasion surprise, but investigations indicate that western white pine seed may remain viable in the ground for five and six years following a burn. In 1914 white pine seed was recovered from duff underneath a 150-year-old stand on Big Creek, Kaniksu National Forest. These seeds were placed immediately in the greenhouse under conditions favorable for germination. The first germination was about 18 months later. Seeds which require 18 months of favorable temperature conditions before germination in the greenhouse might well require four or five seasons under unfavorable germinating conditions in the field.

The second period of germination showed up very strong only on the 1870 and 1910 double burn on Trail Creek. The seed in this case could well have come from trees located from one-half to one mile away to windward. The germination in 1920 on Alder Creek shows a separate and subsequent seeding. Presumably the seed did not come very far or similar conditions would have been found elsewhere. The inference is that the seed was distributed during the fall of 1919, either during or after the big fire, and that the green trees about one-half mile to the south furnished the seed.

Considering only the germination on the single 1910 burns on Trail Creek, there is a more or less sustained restocking on both the exposed and the protected aspects. In most cases this shows a fair germination in 1920, poor in 1921, rising again in 1922, and lowering in 1923. Since there are no green trees of white pine which furnish seed, closer than one-half mile, and as these seedlings appeared promiscuously over the area without reference to the character of the surface, no other deduction seems possible than that here a long range seeding is in progress.

EROSION AND SITE DETERIORATION

Except for steep south slopes, leaching and site deterioration are negligible, or at least are not a factor to be concerned over. Certain deep gullies have been cut on the lower north slope on Alder Creek (pl. IV, B). Here the head gathered by the water, presumably at the time of most rapid snow melting in spring, has cut gullies extending 500 to 600 feet up the slope. Soil, as well as rock, is carried down these gullies into Alder Creek bottom, where the bowlders remain while the finer particles are carried away.

Steep south slopes, however, present a serious situation. Here the burns are so destructive to all vegetation and roots that finer particles of the soil leach out or wash away, leaving a surface covering of fine angular rock and gravel which becomes very hot and very dry in the summer, so much so that natural seedlings, when these do come up, succumb to drought shortly after germination. Even artificial reforestation on such sites is very difficult.

VEGETATION

The following observations are briefly the more outstanding conclusions regarding some phases of succession of

[2] On the Alder Creek area it is not absolutely certain that no germination took place in 1917; there were some seedlings the ages of which were doubtful.

vegetation on different burns. From the 1923 study it appears that the vegetation which has followed the single and double fires on the Deep Creek and Trail Creek areas is but a scanty growth and as such is a benefit rather than a detriment to natural restocking. On severe double burns a definite cycle of succession is evident. It begins with light-seeded willow, everlasting fireweed, goldenrod, and thistle. This series runs its course during the first decade and is then crowded out by snow brush, goat brush, maple, juneberry, huckleberry, ocean spray, ninebark, thimbleberry, snowberry, elderberry, and cherry. Some of these seed in directly after the burn, others spring from sprouts, and still others are seeded in later by birds.

On moist sites much of the original forest vegetation—which includes Coptis, Tiarella, Pyrola, Claytonia, Linnaea, and various ferns and herbaceous plants—takes direct possession of the ground. Evidently the fire in such cases has not burned deeply enough to kill the roots or the seeds. This type of vegetation is always more in evidence on single than on double burns.

The above conditions hold generally for north slopes, high benches, and gentle south and west aspects. On steep south and west slopes, however, the succession is quite different. The lower portions, which become very dry and warm, show a strong tendency toward formation of sod. While this retards erosion, it is thought to deter natural reproduction. Such sites are very dry in midsummer, and the many herbs and shrubs which occur elsewhere and which would be very beneficial as soil builders, checks to erosion, and in furnishing shelter to seedlings, are wanting. The upper south slopes and knolls become, by repeated fires, almost a pure snow brush type. This snow brush is sometimes present before the fire, and sprouts profusely afterwards. It frequently seeds in directly after or during the fire. When the second burn occurs the brush takes almost complete possession by rapid sprouting, as its stout and deep roots have escaped injury. This brush cover later yields its place to the forest, but in the early life of the stand it nurses and protects the scattered young seedlings.

From the standpoint of grazing, these burns must be a distinct disappointment. The vegetation is of poor quality and rather sparse. The sheep would have to travel too much to find sufficient forage. In this respect both the single and the double burns of 1910 and 1919 on the Coeur d'Alene National Forest fall far below the Clearwater National Forest. On the latter, many of the double burns are followed by a wealth of high and dense vegetation which often hinders natural restocking. The profuse and palatable wild pea and the hollyhock, so abundant on the Clearwater Forest, are much less evident on the Coeur d'Alene.

Species of Ribes, which would act as carriers in the event of an invasion of the blister rust disease, occur over the Coeur d'Alene burns, but these are chiefly confined to the watercourses and are generally few and far between on the slopes.

SUMMARY AND CONCLUSIONS

Through analysis of the natural restocking on singly and doubly burned areas on the headwaters of the Coeur d'Alene River in northern Idaho, certain conclusions seem to be warranted. In every instance where a single fire destroyed a mature forest, as in the fires of 1870, 1889, and 1910, the natural restocking of the forest has been prompt, uniform, and complete, and western white pine has made up a goodly proportion of the reproduction. In the case of double burns, however, as instanced by that of 1910 and 1919, restocking is woefully deficient.

The time interval between a first and a second fire greatly influences results, for if fire covers the area a second time before any of the young growth has produced seed, the chances of natural reproduction are much reduced. An area burned in 1870 and again in 1910 showed very good restocking, except on lower south and west aspects. The young forest, which was 35 to 40 years old when destroyed in 1910, had produced seed which evidently helped to restock the area after the 1910 fire. The failure of reproduction on the exposed aspects must be considered as directly due to the action of the two fires in reducing the quality of the site, and in causing extreme and critical conditions for natural reestablishment.

Such double burns, occurring at intervals up to seeding age of the young trees, are followed by heavy grass sod on lower south slopes and dense mats of snow brush on the upper south and west. The grass is considered a hindrance, and the snow brush a help, in natural restocking. Large areas which burned in 1889 and again in 1910 contain scattered seedlings which are nursed along on a very critical site by the prevalent brush.

The outstanding and most extensive double burn of 1910 and 1919 on the Deep Creek area showed very scant new reproduction—from one to two new seedlings per acre on protected north and east aspects, and one or none on exposed aspects. There is much western larch reseeding on northerly aspects from parent trees which survived one or both of these fires. Numerous groups of seedlings 8 to 13 years old occur in patches over this double burn, but only where the force and sweep of the last fire was less intense. After a period of about 20 years these groups should produce seed, which will aid in restocking the double burns. Several groups of mature trees were also found to have survived the two severe fires. These exist either on sharp knolls, on rocky spurs, or in deep draws. They are now supplying seed for restocking of the double burn at distances up to 9 and 11 chains, mainly toward the north and east, in the direction of the prevailing wind.

Close examination of the ages of white pine seedlings found on the various 1910 burns shows that very little germination takes place during the first and second year after the fire, and that the greatest number of white pine seedlings have become established during the fourth, fifth, and sixth years following the burn, probably from seed buried in the soil by rodents previous to the fire. Later seeding is much less, in some years almost nothing, and is evidently due to distribution of the seed, in some cases for distances of one or more miles. An effort to correlate this later seeding with seed crops has not brought positive results, mainly because there is too much uncertainty regarding the local seed production in past seasons.

Both the character and the scantiness of the vegetation observed on the large double burns of 1910 and 1919 indicate very poor grazing values. In most places the vegetation is a help rather than a hindrance to natural reforestation.

Subsequent to the large double burns of 1910 and 1919 there was but slight erosion, which took the form of deep gullying on lower north slopes. The most serious site deterioration was on the steep lower south aspects in the form of leaching or washing away of the finer loam and soil particles, leaving a relatively large proportion of rock and gravel and causing extremes of surface drought and heat detrimental to natural restocking.

On the basis of these and previous studies, as well as from general observation over a period of years, it is believed that there need be no concern about natural restocking after single burns in northern Idaho where forests of seed-bearing age are destroyed. Only one instance of failure of natural restocking has been found thus far namely, on the south aspect of Johnigan Mountain on the Clearwater National Forest. There is, therefore, no necessity for artificial reforestation on single burns in northern Idaho. The situation on large double burns, however, such as those of 1910 and 1919, clamors for thought and action. It would seem to be well to confine all efforts of planting to south and west slopes, in order to get the plants established before the site has deteriorated so far as to render artificial restocking difficult.

The distribution of seed from groups of seed trees is directly applicable in silviculture, for it can safely be assumed that seed dispersal on cut-over areas will be more uniform and effective than that shown on burns, mainly because the physical conditions of the site on logged areas are much less critical for seedlings than are conditions on double burns.

The data on restocking from seed already on the forest floor at the time of the fire are not altogether safe to apply. We need to know more about the source of the seed which gives rise to the first cycle of reproduction on burns, and this can be determined only by the installation of permanent sample plots on fresh and large burns. In the application of the results to cutting practice, the point should be kept in mind that fires in virgin timber burn under different conditions than those on logged land. On logged land there is greater concentration of heat and more complete consumption of duff and other surface material than when virgin forests burn. It may be said, however, that indications are very strong, from the study of both logged and burned areas in the western white pine forests, that the seed which gives rise to the first cycle of natural reproduction is already in the duff at the time the mature seed trees are removed; but this can be proved only by the study of permanent sample plots

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